MODELING THE CD4 DECLINE IN HIV WITH LOW COST PREDICTORS FOR EFFECTIVE HIV MANAGEMENT IN RESOURCE POOR SETTINGS

Dissertation submitted to

The Tamil Nadu Dr. M. G. R. Medical University,

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in partial fulfillment of the award of degree of

MASTER OF PHARMACY

(Pharmaceutical Biotechnology)

Submitted by

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CERTIFICATE

This is to certify that the dissertation entitled **'MODELING THE CD4 DECLINE IN HIV WITH LOW COST PREDICTORS FOR EFFECTIVE HIV MANAGEMENT IN RESOURCE POOR SETTINGS'** being submitted to **The Tamil Nadu Dr. M.G.R. Medical University, Chennai** in partial fulfillment of the **Master of Pharmacy** programme in **Pharmaceutical Biotechnology**, carried out by **Miss K. PUSHPAVALLI** in the Department of Pharmaceutical Biotechnology, College of Pharmacy, SRIPMS, Coimbatore, under my direct guidance and supervision to my fullest satisfaction.

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"No work is accomplished, with optimum refinement; without the support and indulgence of eminent personalities"

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Pushpavalli.K

CONTENTS

INTRODUCTION

Human immunodeficiency virus type1 is a primary cause of AIDS, which is a slow and degenerative disease of human immune system. The pathogenesis of HIV-1 is complex and characterized by interplay of both host and viral factors. Most researchers believe that HIV originated in sub-Saharan Africa during 20th century[1] and is now a pandemic, with an estimated 33.2 million people now living with the disease worldwide. As of January 2006, the United Nations Programme on HIV/AIDS (UNAIDS) and World Health Organization (WHO) estimated that AIDS has killed more than 25 million people since it was first recognized on June 5, 19812, making it one of the most destructive epidemics in recorded history[2].

2.1 HIV-1

HIV-1 is a lentivirus belonging to the retrovirus family. The virus is diploid and contains two plus-stranded RNA copies of its genome. The approximately 9 kb RNA genome encodes at least 9 proteins, Gag, Pol, Env, Tat, Rev, Nef, Vif, Vpu and Vpr[3]

Three major classes of HIV-1 have emerged: M (main), N (new), and O (outlier)[4]. Among M group viruses, which account for >90% of HIV infections worldwide, there are 9 subtypes, called clades, designated by the letters A-D, F-H, J, and K, as well as many recombinant forms.

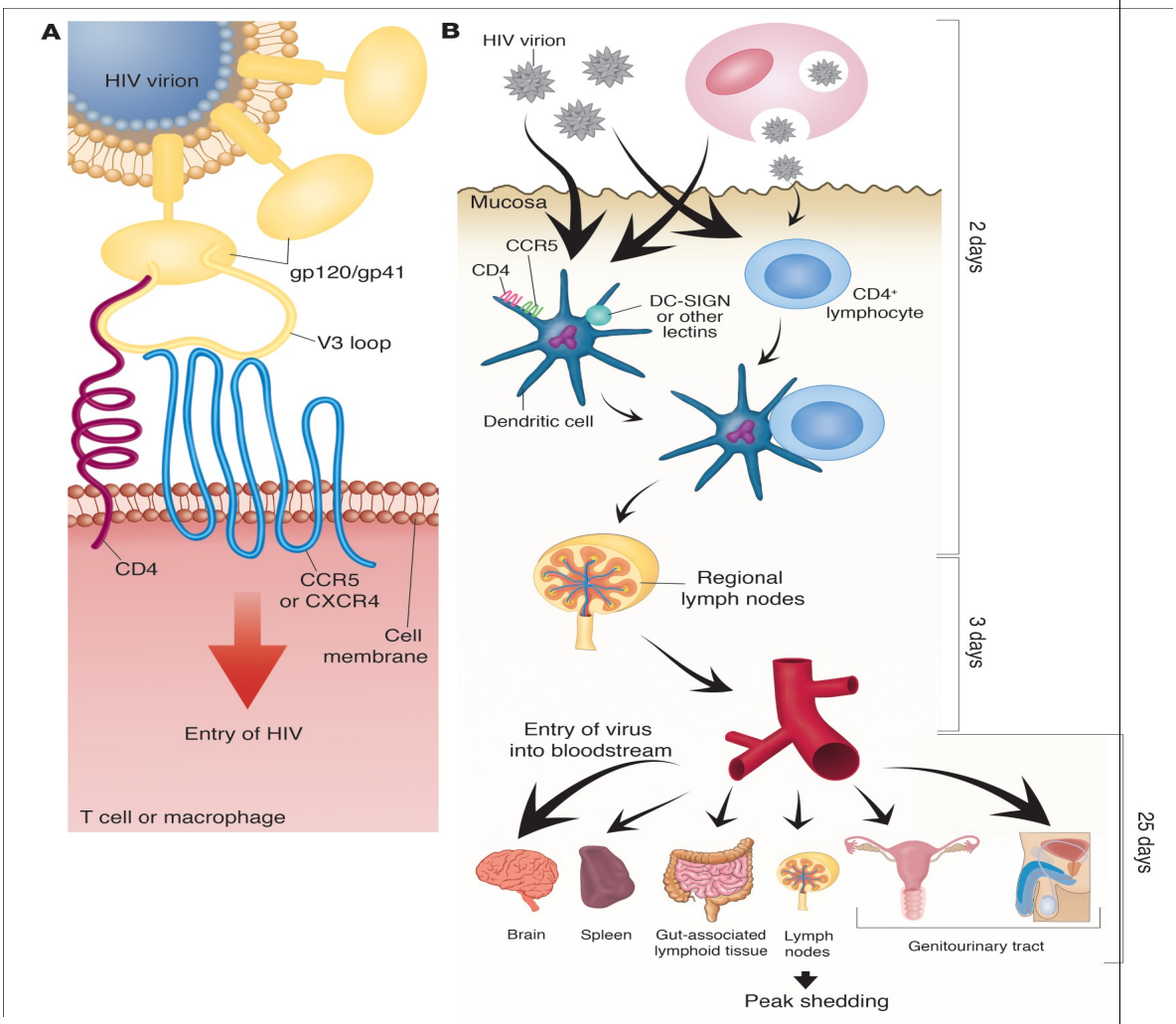
2.2 HIV and genetic variation[5]

Virus type	Sub type	Predominant area
HIV-1 M	A	Africa, Eastern Europe
	B	Europe,North America,Austrilia,Thailand
	C	Southern Africa, India
	D	East and Central Africa
	E	Thailand
O\N		West Africa

2.3 Pathogenesis

Infection is transmitted when the virus enters the blood or tissues of a person and comes into contact with a suitable host cell principally CD4 lymphocyte. B lymphocyte, monocytes, macrophages, including specialized macrophages such as alveolar macrophage in lungs and Langerhans cells in the dermis, glial cells and microglia in the CNS are also susceptible. The infection spreads to the lymphatic tissue that contains follicular dentritic cells that may act as storage place for latent viruses. Over time, virus replication leads to the immune system[6].

Fig:1

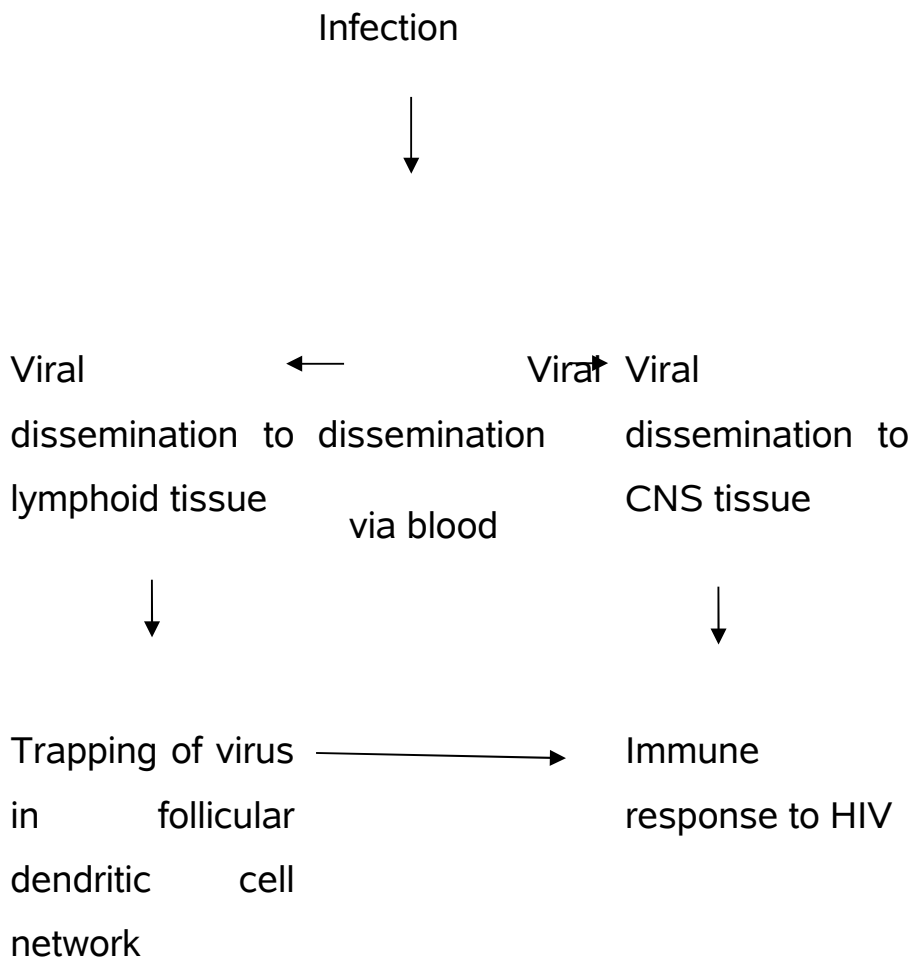


HIV transmission and the establishment of HIV reservoirs. (A) Interactions of HIV envelope glycoprotein, CD4, and CCR5 or CXCR4 co receptors Trigger fusion and entry of HIV. (B) Outline of the sequence and time course of events involved in viral dissemination.

2.4 Immunology

Individuals infected with HIV show both cellular and humoral (antibody) immune responses to the virus, but these responses are unable to prevent the ultimate progression of disease in the great majority of infected individuals. Cellular responses are mediated by CTLs (CD8 cells) and helper T lymphocytes (CD4 cells)[8].

Fig:2 **Viral Pathogenesis and immune response[9]**



↓

Sequestration of virus in lymph nodes

↓

Virus actively replicating in lymphoid tissue, CD4 cells and follicular dendritic cells

↓

Disruption of follicular dendritic cell network, with increasing 'escape of virus' → Increased demands on immune response

↓

Destruction of the immune system due to constant viral

↓

replication

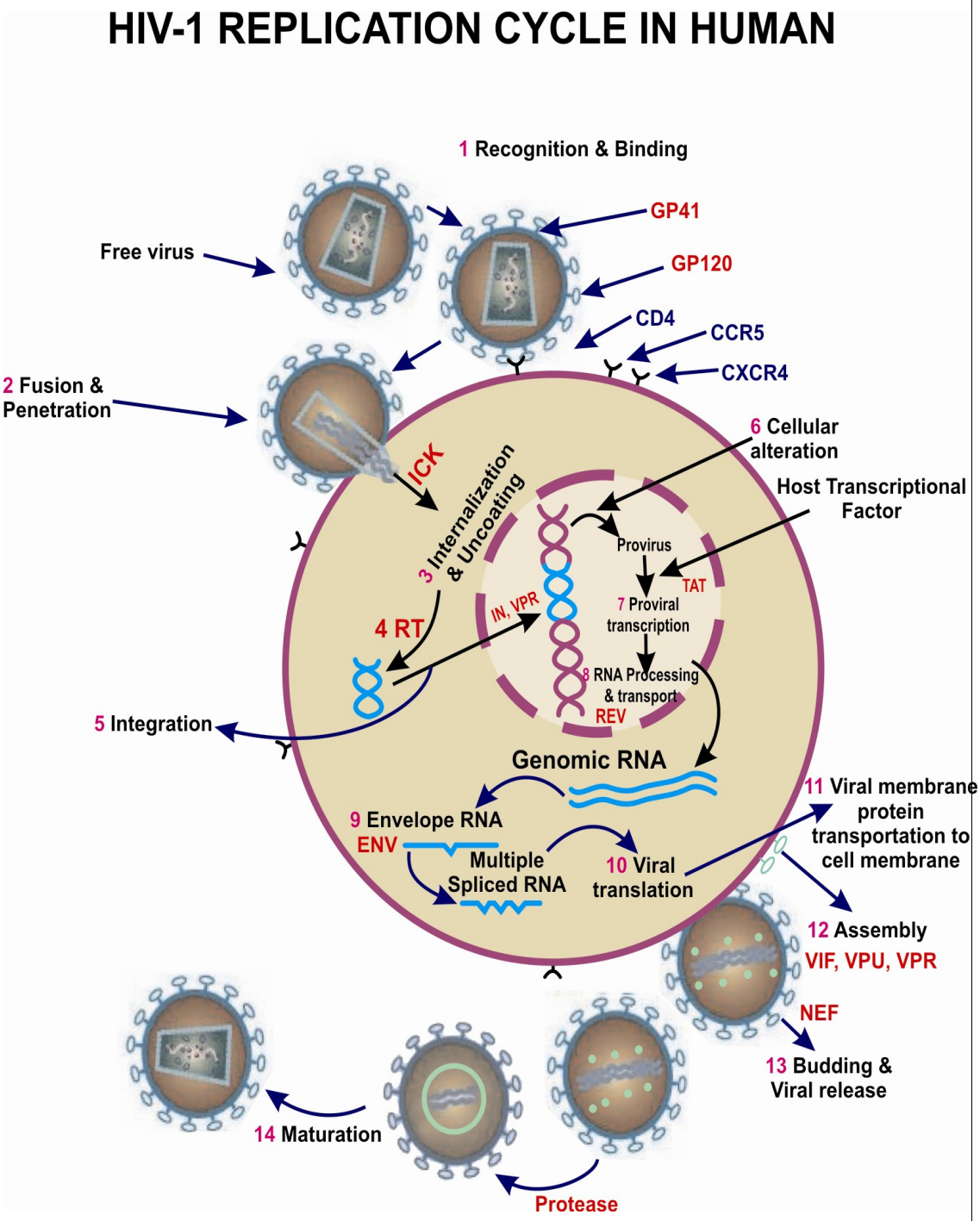
Increased risk of infections and tumors

Appearance of clinical symptoms

AIDS

2.5 HIV Replication cycle[10, 11, 12]

Fig:3



Summary of HIV life cycle

1. Free virus enters into blood stream of human via various transmissions, and then recognizes

the site of infection and binds to it.

2. Fused with cell membrane and penetrate to cytoplasm

3. Internalization and uncoating supported by various host factors

4. Single standard viral RNA transcribed into double standard DNA by reverse transcriptase

5. Integrate their double standard DNA into host genome influenced by IN and VPR

6. Proviral formation

7. Proviral transcription by TAT

8. RNA of virus processed by REV and transported into cytoplasm

9. Formation of unspliced product of genomic RNA influenced by ENV.

10. Viral translation

11. Viral membrane protein transported to cell membrane

12. Assembling of viral proteins in immature virus

13. Virion release through budding process influenced by NEF

14. Maturation of virus by protease enzyme

Each step in replication cycle is equally important to make an infective virion and thus each step acts as novel target for antiviral strategies .Focusing the molecular strategies which delays the progression or block replication of HIV-1 infected individual's gains paramount importance.

Table:1 **Molecular strategies against targets in HIV replication cycle**[3, 11, 13, 14, 15]

Targets	Strategy against target	Drugs
1	1. chemokine ligand mediates receptor blokade	RANTES 1.AOP 2.PSC
	2.Antisence RNA	VRX4-96
	3.Ribozymes	-
	4.SiRNA(0.5 Kb dsRNA CD4 domain of SU)	-
	5.Chimeric \fusion protein	PRO 542
	6.Intracellular antibody (sFUS)	Cynovirin-11KDa protein isolated from

		cyano bacteria
	7.Monoclonal antibody	Mab 12G5
		Anti ECL 2
		TNX-355
	A.Receptor blocking	
	1.chemicals	AMD 3100
		AK 602
		AMD 070
		INCB 9471
2		Maraviroc *
		Enfuvirtide *
	2.Mab	PRO 140
		HGS 0004
	B.GP 120 inhibition	BMS 378806
4	1.NRTI	Lamivudine *
		Abacavir *
		Zidovudine *

		Stavudine *
		Didanosine *
		Emtricitabine *
		Tenofovir *
		Apricitabine
		Amdoxovir
		Dioxolan
		ethyminedine
		Elvucitabine
		KPUI 461
		MIV 210
		RAcivir
	2.NNRTI	Delavirdine *
		Efavirenz *
		Nevirapine *
		(+)calanolide
		BILR 355 B
		Etravirine
		MIV 150

			Rilpivirine
		3.Ribozymes	-
		4.RNA decoy	tRNA 3 Lys mutant
		5.Anti RT dimerization	Small peptide 15-19 AA
		6.RNA aptamer	-
		7.Anti sense	-
5		1.Irreversible noncompetitive enzyme inhibition	Dicaffeoyl quinic acids
		2.other chemicals	2,4 dioxo butanoic acid analog Reltegravir GSK 364735 Elvitegravir
		3.Dinudotide	Pdc pISodv
		4.sFU	-
		5.SiRNA	-

7.	1.Antisense RNA	HGTV43
	2.RNA decoys	TAR decoys
	3.Si RNA	Tat SiRNA Vs NF bp 65
	4.Transdominant negative protein	Tat mutant
	5.Ribozymes	RR Z2
	6.Fusion protein	-
	7.Mab	BI-201
		Benzodiazepine oligo pyrrole hybrids
	8.chemical agent	Aromatic polyamide carrying halogen
		1.TAPB-Br
		2.TAPP-Br
8.	1.TNP	Rev M10
		Rev 38
		Sam 68 mutant

		PRE decoys	
	2.RNA decoys	C41 nucleotide	
		Rre sciIAB	
		RNA decoy	
	3.SFU	NES specific SFU	
	4.Antisense	Synthetic phosphorothiosate oligodeoxy nucleotide	
	5.RNA aptamer	-	
		Leptomycin B	
	6.Antibiotic	NUP 214\CAN	
		Delte-CAN	
	7.Fusion protein	NSIRM –Rev mutant	
	8.intercalating dye	Pyronin Y	
		Diphenyl furan	
9.	1.Antisense	Anti sense to U3 LTR gag –Env	5'
		Env region	
	2.Gag TNPS	-	

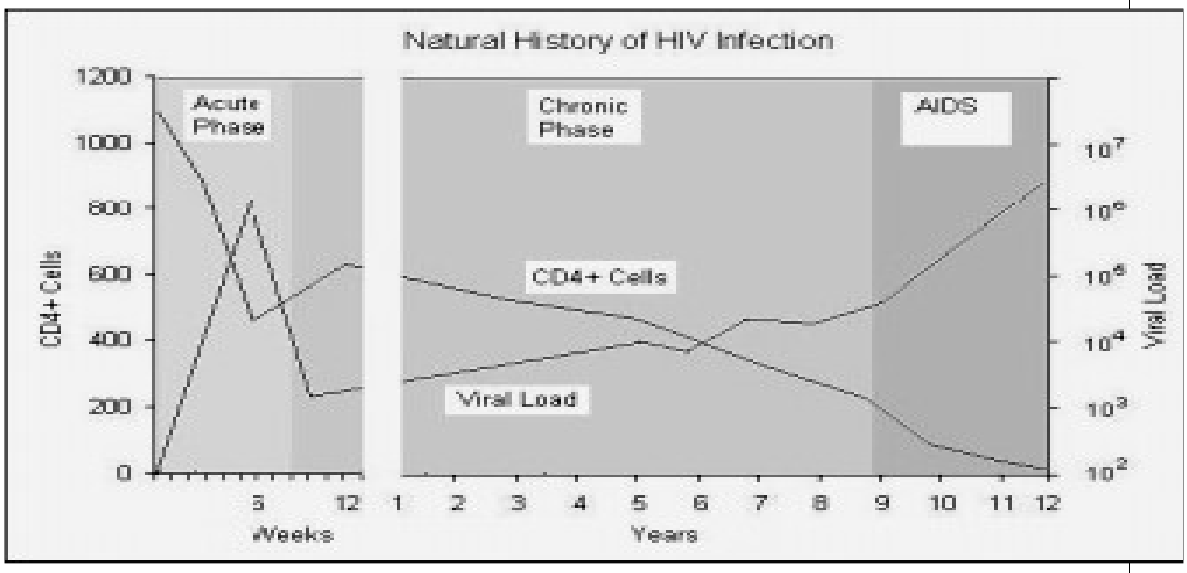
	3.sFU	SFU 105
	4.Anti intracellur protein	Poly arginine peptide
12.	1.Anti sense	RNA 1.43kb to psi-gag region
	2.Ribozymes	-
	3.RNA decoys	-
13	1.Nef TNPs	-
	2.Anti sense	-
	3.Ribozymes	-
14.	1.Gag TNP	-
	2.Anti sense	-
	3.Protease inhibitor	Saquinavir *
		Indinavir *
		Ritonavir *
		Nelfinavir *
		Amprenavir *
		Lopinavir *

	Atazanavir *
	Lopinavir *
	Atazanavir *
	Daruanvir *
	Tipnavir *
	Darunavir *
	Fosampnonavir *
	PPL-100
4.Maturation inhibitor	Bevirrimat *

2.6 Natural progression of HIV-1

Natural history of infection encompasses an acute phase that lasts months, followed by an early /clinically latent phase that typically 3-10 years, and ultimately by the immune collapse characterized by AIDS[7].

Fig:4



Four stages of HIV infection can be described[9]

i) Primary infection: Infection with HIV results in rapid proliferation of the virus in blood and lymph nodes. The infected person may experience a seroconversion illness, which usually resolves within weeks. The CD4 cell count declines rapidly before virus is controlled by the immune system, whereupon the count returns to near normal.

ii) Early immune deficiency (CD4 cell count>500/ML):During this phase the immune system has controlled the virus, which is largely restricted to lymphoid tissue. In this phase, damage inflicted by the virus is limited to the regenerative capacity of the immune system and people with HIV are usually without symptoms.

iii) Intermediate immune deficiency (CD4 cell count 200-500/ML): Viral replication is very high and CD4 cell turnover is rapid. Subtle signs and symptoms indicating compromise of immune system begin to appear.

iv) Advance Immune Deficiency (CD4 cell count <200/ML): The virus which proliferates throughout the body overcomes the immune system. Major opportunistic infections and malignancies become increasingly common and require increasing medical intervention

Table:2 Natural History of HIV Infection in adult

EXPOSURE TO HIV INFECTION	**Seroconversion illness**	**Asymptomatic period**	**Subtle Symptoms and Signs of Immunodeficiency**	**Full Blown AIDS Symptoms and Signs of opportunistic Infections**

ANTIBODY TITRE AND INFECTIVITY	**Not Detectable** **(Window Period)** **Highly Infectious**	**Detectable antibodies** **Infectious**	**Detectable antibodies** **Infectious**	**Antibodies may or may not be detectable** **Highly Infectious**
TIME PERIOD	**12 Weeks**	**3-5 years**	**2-3 years**	**1-2 years**
STAGE OF HIV DISEASE	**Primary Infection** **(CD4 cell count near**	**Early Immunodeficiency** **(CD4 cell count >500**	**Intermediate immunodeficiency** **(CD4 cell count**	**Terminal illness** **(CD4**

	normal)	**cells/μ l)**	**<500** **>200 cells /μl**	**cell coun t <200 cell / μl)**

2.7 Classification of HIV Disease

HIV damages the immune system, leaving the infected person vulnerable to a variety of infections (called "opportunistic" infections to indicate that they arise in the setting of immune impairment). The effect of HIV on the immune system is monitored by measuring the CD4 (helper) lymphocyte count in the blood. A normal CD4 count (between approximately 600 and 1,200 cells/μL) indicates that the immune system has not undergone sufficient damage to put the individual at risk for opportunistic illness

1993 Revised Classification System for HIV Infection and Expanded Surveillance Case Definition for AIDS Among Adolescents and Adults by **CDC**

CD4+ T-Lymphocyte Categories

The three CD4+ T-lymphocyte categories are defined as follows:

Category 1: greater than or equal to 500 cells/mL

Category 2: 200-499 cells/uL

Category 3: less than 200 cells/uL

Clinical Categories

The clinical categories of HIV infection are defined as follows:

Category A

Asymptomatic HIV infection

Persistent generalized lymphadenopathy

Acute (primary) HIV infection with accompanying illness or history of acute HIV infection

Category B

Bacillary angiomatosis

Candidiasis, oropharyngeal (thrush)

Candidiasis, vulvovaginal; persistent, frequent, or poorly responsive to therapy

Cervical dysplasia (moderate or severe)/cervical carcinoma in situ

Constitutional symptoms, such as fever (38.5 C) or diarrhea lasting greater than 1 month

Hairy leukoplakia, oral

Herpes zoster (shingles), involving at least two distinct episodes or more than one dermatome

Idiopathic thrombocytopenic purpura

Listeriosis

Pelvic inflammatory disease, particularly if complicated by tubo-ovarian abscess

Peripheral neuropathy

Category C

Candidiasis of bronchi, trachea, or lungs

Candidiasis, esophageal

Cervical cancer, invasive *

Coccidioidomycosis, disseminated or extrapulmonary

Cryptococcosis, extra pulmonary

Cryptosporidiosis, chronic intestinal (greater than 1 month's duration)

Cytomegalovirus disease (other than liver, spleen, or nodes)

Cytomegalovirus retinitis (with loss of vision)

Encephalopathy, HIV-related

Herpes simplex: chronic ulcer(s) (greater than 1 month's duration); or bronchitis, pneumonitis, or esophagitis

Histoplasmosis, disseminated or extra pulmonary

Isosporiasis, chronic intestinal (greater than 1 month's duration)

Kaposi's sarcoma

Lymphoma, Burkitt's (or equivalent term)

Lymphoma, immunoblastic (or equivalent term)

Lymphoma, primary, of brain

Mycobacterium avium complex or M. kansasii, disseminated or extra pulmonary

Mycobacterium tuberculosis, any site (pulmonary * or extra pulmonary)

Mycobacterium, other species or unidentified species, disseminated or extra pulmonary

Pneumocystis carinii pneumonia

Pneumonia, recurrent *

Progressive multifocal leukoencephalopathy

Salmonella septicemia, recurrent

Toxoplasmosis of brain

Wasting syndrome due to HIV

The World Health Organization has developed a clinical staging system for HIV infection. This system relies more heavily on clinical rather than laboratory evaluation, and has been used widely in resource-constrained areas where laboratory testing is not widely available.

WHO STAGING SYSTEM FOR HIV INFECTION AND DISEASE IN ADULTS AND ADOLESCENTS

Clinical Stage I:

Asymptomatic

Persistent generalized lymphadenopathy (PGL)

Performance scale 1: Asymptomatic, normal activity

Clinical Stage II:

Weight loss, < 10% of body weight

Minor mucocutaneous manifestations (seborrheic dermatitis, prurigo, fungal nail

infections, recurrent oral ulcerations, angular cheilitis)

Herpes Zoster, within the last 5 years

Recurrent upper respiratory tract infections (i.e., bacterial sinusitis) and/or

Performance scale 2: symptomatic, normal activity

Clinical Stage III:

Weight loss, > 10% of body weight

Unexplained chronic diarrhoea, > 1 month

Unexplained prolonged fever (intermittent or constant), > 1 month

Oral candidiasis (thrush)

Oral hairy leukoplakia.

Pulmonary tuberculosis, within the past year

Severe bacterial infections (i.e., pneumonia, pyomyositis) and/or performance scale

3: bed-ridden, < 50% of the day during the last month

Clinical Stage IV:

HIV wasting syndrome, as defined by CDC

Pneumocystis carinii pneumonia

Toxoplasmosis of the brain

Cryptosporidiosis with diarrhoea, > 1 month

Cryptococcosis, extrapulmonary

Cytomegalovirus (CMV) disease of an organ other than liver, spleen or lymph nodes

Herpes simplex virus (HSV) infection, mucocutaneous > 1 month, or visceral any

du ration

Progressive multifocal leukoencephalopathy (PML)

Any disseminated endemic mycosis (i.e. histoplasmosis, coccidioidomycosis)

Candidiasis of the oesophagus, trachea, bronchi or lungs

Atypical mycobacteriosis, disseminated

Non-typhoid Salmonella septicaemia

Extrapulmonary tuberculosis

Lymphoma

Kaposi's sarcoma (KS)

HIV encephalopathy, as defined by CDC

And/or Performance scale 4: bed-ridden, > 50% of the day during the last month

(Note: both definitive and presumptive diagnoses are acceptable.)

2.8 Epidemiology

There are currently approximately 33.2 million persons living with HIV\AIDS. Among them adult prevalence is 0.8%. As per 2007 WHO report, adult and children deaths due to AIDS are 2.1% million, and adult and children newly infected with HIV is 2.5 million.

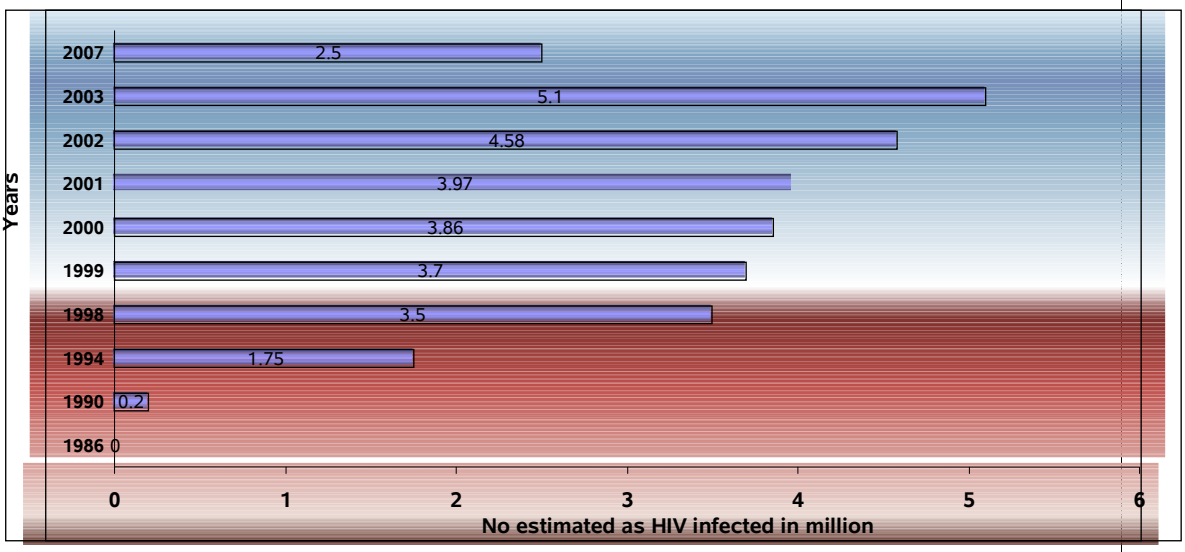
In India, the adult prevalence % is 0.36; total prevalence is 0.9%.Total population of HIV in India is 2.5 million at 2007

Adult prevalence % in different states of India is[19],

Table:3

State	Prevalence %
Andrapradesh, Nagaland, Manipur	1-2%
Karnataka, Maharashtra, West Bengal, Mizoram	0.5-1%
Tamil Nadu	0.2-0.5%
Rest	0-0.2%

Fig:5 HIV population of India in millions



India accounts for 10% of global HIV burden and 65% of that in South and

South East Asia.

2.9 Treatment

Currently, all FDA-approved antiretroviral medications work by inhibiting 1 of 3 steps in the life cycle of HIV:

1. Blocking the reverse transcriptase (RT) enzyme. The RT enzyme is used by the virus to convert its RNA into DNA after the virus enters the cell but before it enters the nucleus. All nucleoside and nucleotide analogues as well as NNRTIs function by interfering with the activity of this enzyme.

2. Blocking the protease enzyme. Protease inhibitors, as their name indicates, inhibit the action of the HIV protease, namely, cleaving protein products of the viral structural genes into the functional subunits needed to create new infectious virions.
3. Inhibiting fusion of the viral and host membranes. By attaching itself to the HIV envelope glycoprotein gp41, fusion inhibitors prevent formation of the "hairpin" structure required for fusion of the HIV and host cell membranes, and thus prevent viral entry into the host cell.

These drugs are classified as[21],

a – NRTI, b - NNRTI, c – PI, d – FI

2.9.1 Selection of ART

Combination of these drugs are possible, termed as HAART, it could exert more effectiveness. Selection of ART regimens for programmes and individual patients should consider; potency, frequency of dosage, side effects, maintance of future treatment options, the anticipated adherence of the patient population to a regimen, need for storage, concurrent conditions, the potential for resistant viral strains and cost access.

2.9.1.1 Possible HAART combinations

Table:4

No of possibilities	Choice of combinations
1	A
2	2a+b
3	2a+c
4	3a
5	2a+2b
6	A+b+c
7	B+c
8	A+b+c+d
9	c\r alone
10	4a
11	3a+b

Among these,

Current recommendation	2, 3, 4
Not possible category (already trailed) but some cases recommends e.g. failure in 2a+c	5, 6,and 11
Under investigation	7, 8 9, 10

2.9.1.2 Current recommended HAART regimens

Clinicians are recommended to construct a regimen by choosing one component from Column A + one component from Column B.

Table:5

	Column A		Column B
	NNRTI	PI	2-NRTI
Preferred (alphabetical order)	Efavirenz **(AII)**	Atazanavir + ritonavir **(AIII)** Fosamprenavir + ritonavir BID **(AII)** Lopinavir/ritonavir BID **(AII)**	Tenofovir/emtricitabine **(AII)** Zidovudine/lamivudine **(AII)**
Alternative (alphabetical order)	Nevirapine **(BII)**	Atazanavir (unboosted) **(BII)** Fosamprenavir (unboosted) **(BII)** Fosamprenavir + ritonavir once daily **(BII)** Lopinavir/ritonavir once daily **(BII)**	Abacavir/lamivudine **(BII)** Didanosine + lamivudine **(BII)**

2.9.2 Initiation of HAART[22, 23]

Optimal timing of ART initiation for persons with HIV infection is of great clinical and public health importance. There is strong evidence supporting the use of the CD4 T cell count as the major determinant in initiating therapy. The plasma HIV RNA level remains an independent predictor of clinical outcome in patients who do not receive ART and is the best available guide for monitoring the effectiveness of ART.

2.9.2.1 Current guidelines[24,25, 26]

Current guidelines from the international AIDS society-USA (IAS-USA), DHHS, BHIVA all recommends ART for individuals with symptomatic HIV infection. These established guidelines uniformly recommend initiating ART before the CD4 count is < 200 cells\mm3.

WHO recommends that in ART programmes in resource-limited settings HIV infected adolescent and adults should start ART when

they have clinical AIDS, regardless of CD4 count, When TLC can be assessed, in addition people with WHO stage 2 or stage 3 HIV diseases should be offered treatment when CD4 counts are available, all HIV infected people with less than 200 CD4 cells\mm3 should be offered treatment.

2.9.3 Acute HIV treatment[27]

Most individuals with acute HIV infection are symptomatic and could be targeted for early therapy. Information on long-term outcomes of treatment for acute HIV infection is limited. Possible benefits of early therapy include: less severe acute disease, lower initial viral "set point" with potential slower disease progression, preservation of immune function, and possible reduction in viral transmission. On the other hand, potential risks include drug toxicities and, if virus is not fully suppressed, drug resistance that limits further treatment options. Based on limited available data, some researches found that, treating HIV during the acute HIV infection stage may boost the immune system and slow the progression of HIV disease.

One study followed HIV positive persons who started HAART in the acute infection stage. This person had significantly better viral load and CD4 counts, compared to HIV positive person who began HAART at a later stage.

2.9.4 Survival benefits of HAART[30, 31]

The time from first diagnosis of AIDS to death has been characterized separately from the incubation time from infection to AIDS as AIDS survival time. Improved median survival of AIDS patients was observed on HAART. In the Multicenter AIDS Cohort Study, the estimated median time from seroconversion to death for a person infected at age 30 was associated with use of combined therapy and increased nearly 2 years.

In another one study, found that median survival time increased from 19 to 30 months among all AIDS patients over age 35 years with a CD4 lymphocyte count less than 100 cells/µl and receiving antiretroviral therapy.

2.10 Laboratory monitoring of HIV

HIV infection is spreading rapidly in India. The infection can only be detected by laboratory tests, as there is a long asymptomatic period when the individual is infectious and can spread disease but has no specific symptoms or signs of disease. The role of the laboratory is very important and it is essential that the highest standards are not only maintained in each laboratory but are also regularly monitored.

Laboratory surrogate markers of HIV infection are needed to provide information about the stages and course of disease, especially in the years prior to the development of clinical signs and symptoms of immune disorder, e.g. OP infections or neoplasia. Changes in immune

parameters also reflect damage to the host caused by HIV infection and indicate pathogenic mechanisms of disease.

2.10.1 Classification of surrogate markers based on disease pathogenesis

In clinical context “surrogate” implies some responsibility e.g. an ability to contribute substantial information about clinical prognosis. A separate system for classifying the stages of surrogate marker evaluation has been proposed by Mildvan et al ,this involves progress in staging from detecting of HIV infection, to relation to prognosis and finally to relevance to therapeutic benefit.

Table:6

I. VIRAL FEATURES	P^{24} AG ASSAY HIV-1 RNA LEVEL BY PCR, BDNA, NABA
II immunological features	Cd7 lymphocyte count CD4:CD8 CD4% IL-2R TNF-α

	INF-γ IL-4 MHC-1R
III Immune system activation	Ig G Ig A elavation CD38 expression

But the cost of monitoring is prohibitively high. As antiretroviral therapy is becoming more affordable and accessible, inexpensive laboratory tests are also needed to monitor the progression of disease in HIV infected individuals, living in resource-limited environments most heavily impacted by the epidemic. Low cost predictors include serum albumin, BMI, TLC, DTH reaction, ALT, AST measurements and HGB measurement.

2.10.2 Parameters measured in HIV progression and their significance[32, 33, 34, 35]

Table:7

Parameters	Normal condition	HIV-AIDS	Other disease
1.CD4 count in cells\mm3	CD4 marker is exclusively specific marker in T-cells for HIV particle	Immunological detoriation	-
2.CD4:CD8	"		
3.CD4%	"		
4.VL in copies\pl(positive)	-	HIV-factors	-
5. P^{24}Ag (positive)	Measures the viral capsid (core) P^{24} protein, directly reflects the amount of antigen persist in our		

	body.		
6. C-reactive protein (negative)	Reflects activation/ production of IL-4	Immunological alteration	Atherosclerosis, cancer, end stage renal disease
7.β2Microglobulin in mg\dl(positive)	Reflects activation of MHC I K		Cancer, renal impairment, all immuno deficiency status
8.S neopterin in ng\ml(positive)	Reflects more production of IFN -γ		TB, Kindney graft rejection, viral, fungal, bacterial infections, aseptic meningoencephalitis, collegen vascular disease, malignancies
9.s IL -2R in (positive) u/ml	Reflects more production of IL-2		Cancer
10. S-TNFα2RI in ng/ml(positive)	Reflects moreproduction of TNF-α		Cancer
11. S-albumin %g/l(negative)	Decrease their level where	Severe liver dysfunction on ARC cocci	So many hepatic impaired condition

	impaired liver function	dioidomycosis hepatitis- co-infection	
12. ALT,AST (negative)	Reflects liver function		
13.Hb(negative)	Reflects anemic condition	Bone marrow depression, severe anemic condition Neutropenia, malnutrition, malabsorption, myelodysplasia Cytomegaloviral infection, mycobacterium avium complex, crytocococcus, Histoplasmosis, drug induced toxicity and HIV factor,	Malaria, folate deficiency, iron deficiency, hook worm infection, α thalassaemia, TB
14. Platelet count(negative)	Reflects thromobolytic function	Thrombocytopenia purpurae	Common for physical destruction
15. Haemotocrit	Reflects total	Hematological	

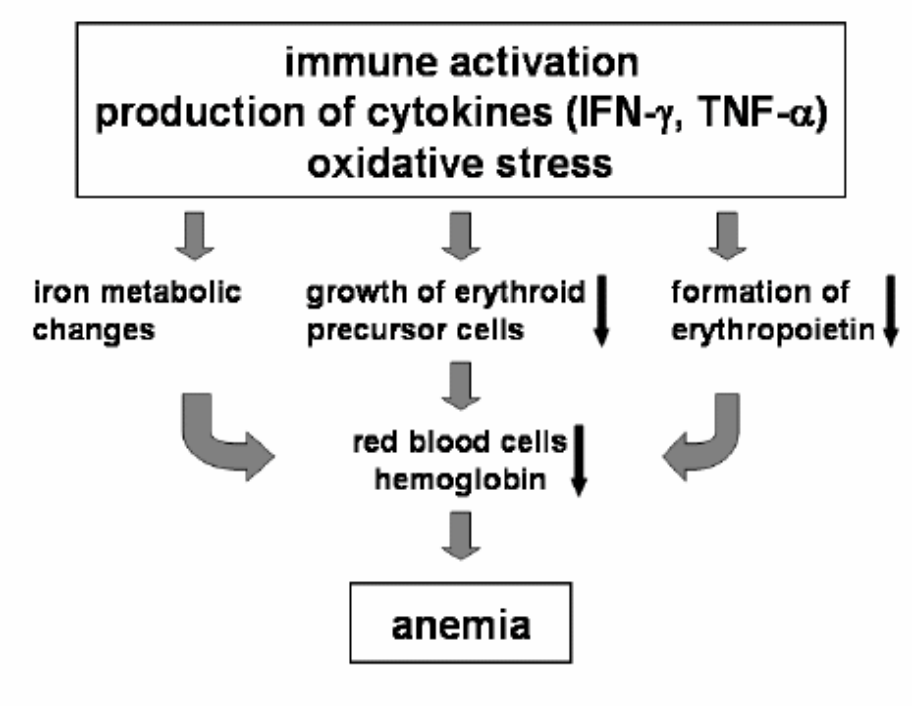
(negative)	red blood cell volume and their status	complication	
16. TLC(negative)	Reflects lymphocyte% multiplied with WBC	leucopenia, lymphopenia	
17. BMI (low)	Reflects alteration in growth factor	Severe weight loss (>10%)	TB, Typhoid, cholera(chronic) and other amoebic conditions

2.11 Impact of anemia in HIV progression[36,37]

Clinical assessment prior to the initiation of ART includes documentation of past medical history, identification of current and past HIV related illness, identification of co morbidities, medications in use and current symptoms and signs. Clinical course, co morbidity and pattern of OI vary from patient to patient and from country to country. But anemic co morbidity is common to all.

Anemia is frequently experienced by HIV positive individuals especially women. Prevalence of anemia in asymptomatic period is 30%, and in clinical AIDS is 80-90%. HIV related anemia can involve multiple casual mechanisms, and often several of these are operative in the same patient at the same time. These causal mechanisms include HIV itself, which can induce chronic inflammation, and slow red blood cell production. In addition, drug toxicities, OI, malabsorption, syndromes leading to folate or vitamin B12 deficiency, blood loss, iron deficiency, lymphoma and other AIDS-related malignancies can all cause or contribute to anemia

Fig:6



Anemia has been associated with decreased quality of life and decreased survival, has been shown to be a strong independent predictor of disease progression and death. Even modest (1g\dL) changes in HGB may associate with a significant increased risk of death.

After HAART was introduced, the prevalence of anemia in HIV infection declined. With increased use of HAART, we have witnessed a decline in OI with malignance and infection-related malabsorption syndrome. Also, HAART may reduce levels of HIV chronic inflammation. All of these factors can improve anemia, and are most likely responsible for the decrease in cases of severe anemia noted during the HAART era. Recovery

from anemia is associated with improved survival and that treatment of mild-moderate anemia in HIV patients enhances functional ability as well as improves quality of life, is of important to both patients and treating physicians. Taken together, this information underscores the continuing importance of monitoring for anemia and maintaining normal HGB levels as a treatment goal, even as ART continue to improve.

OBJECTIVE OF STUDY

1. To integrate the different ways of HIV classification and to develop a classification model which will be more relevant for resource limited settings.
2. To study the interrelationship between the cheaply available markers, (Hb & TLC) with CD4 and to develop a model for "CD4 decline in HIV" for therapeutic decision making.

3. To derive an algorithm based on HGB and TLC measurement for

guide to initiation Of ART

4. To review the molecular strategies against target in HIV replication cycle

3. MATERIALS AND METHODS

3.1 **Instruments Required**

(1) Sysmex Kx-21 – Automatic Blood Content Analyzer.

(2)Flow Cytometer- Becton Dickinson _ CD4 Counter

3.2. Protocol for Complete blood Count

a)**Sample:**

HIV patients Blood (iv source)

b)**Diluation**:

20µl blood diluted with 300µl cell pack which contains, HGB/WBC lyse reagent – Stromolyser, cell clean- detergent and diluent by machine itself.

c)**Detection:**

HGB Detected by photo electric method using 540nm Principle behind it is Cyanomethaemoglobin method.

d)**Determination of HGB**:

Direct reading from report.

e)**Determination of TLC:**

Multiply the WBC with lymphocyte % obtained from reading.

3.3. Protocol for CD4 count

a)**sample :**

Peripheral blood of HIV patients .collect into EDTA coated tubes (367856,Becton and Dickinson).Mix thoroughly with the anti coagulant by gently inverting the tube .about 8-10 mts

b) **Cell staining :**

Add 20µl of antibody (stand)to 50µl of blood sample gently mix it by vortexer

Incubate the sample in the dark for 15mts

c)**Fixation and rbc analysis**:

450µl of IX lysis buffer to each sample.vortex it and incubate for 15mts.

Add 25 µl of fluorescent beads to each sample.

d)**Calibration of the flow cytometer**

e**)Detection:** - FACS method

f)**Determination of CD4** = Gated cell count × Bead count per test

Gated bead count Test volume

3.4. Limitation and precaution of study

Handling of HIV patients blood is risk for unskilled persons those who are not practiced with laboratory equipments

Modes of exposure to HIV in the laboratory

LAB PROCEDURE	METHODS OF TRANSMISSION
Collection of blood	Needle stick injury ,Broken specimen container, Blood contamination of hand with skin lesions or breach Contaminated exterior of the container
Transfer of specimen cleaning/washing	Punctural contamination of skin from, contaminated glass ware sharps contaminated work surface

Transport of specimen to distant laboratory	Broken or leaking container

Similiarly all instruments and other equipments that have come into contact with blood are assumed to be potentially contaminated with blood borne pathogens and must be properly handled,cleaned or safely disposed off.

METHODOLOGY

3.5. Data collection

Collection of data from out sources

Data of demographic, general, clinical characteristics and laboratory profile of HIV positive patients in all stages, collected and reviewed from net sources to find out the changes of the marker trend in all stages of HIV and analyzed their symptoms persist in different stages of disease.

Collection of Existing data

Comparison of results obtained from analysis of net sources with experimental work or existing work is essential for conclude the research work. Due to limitation and precaution of handling of HIV patient's blood, existing work collected for further analysis. Data of 50 HIV +ve patients include general characteristics, clinical profile and lab parameters collected from their records in all departments in 500 bedded multi speciality private tertiary care teaching hospital.

3.6. Data analysis

B. Data analysis

- The data mainly laboratory profile, symptoms of HIV, and other co morbidities, and immunopathology from different sources compared and derived the trend for CD4, VL changes in acute phase, chronic phase and AIDS phase. Also derived the trend for HGB and TLC decline in HIV progression.

- Subgrouped the 3 stages of AIDS into further based upon changes in marker trend, and symptom. Classified the HIV progression into a new model which represents the integrated form of changes in marker trend, symptoms, CDC classification and WHO classification system.

- Correlation between the all surrogate markers which are recognized as predictors of HIV progression is studied and compared their significance in prediction of HIV in resource poor settings.

- Trend for changes of markers level (CD4 count, VL, HGB and TLC) in HIV infection were derived by regression analysis.

- Analysed the characteristics (Age, group, sex, co morbidity prevalence), clinical profile (frequently observed symptoms) and laboratory profile (HGB, TLC, CD4) of study population

from existing data and classified them into different category based upon their symptoms.

C. **Prediction**

- Predicted the CD4 for study population by,

  Correlation of TLC with CD4 count and HGB level with CD4 count, and found out the significance of combination of these 2 models

- Compared the predicted CD4 with actual CD4 of study population

  and find out the significance and outcomes of symptom based

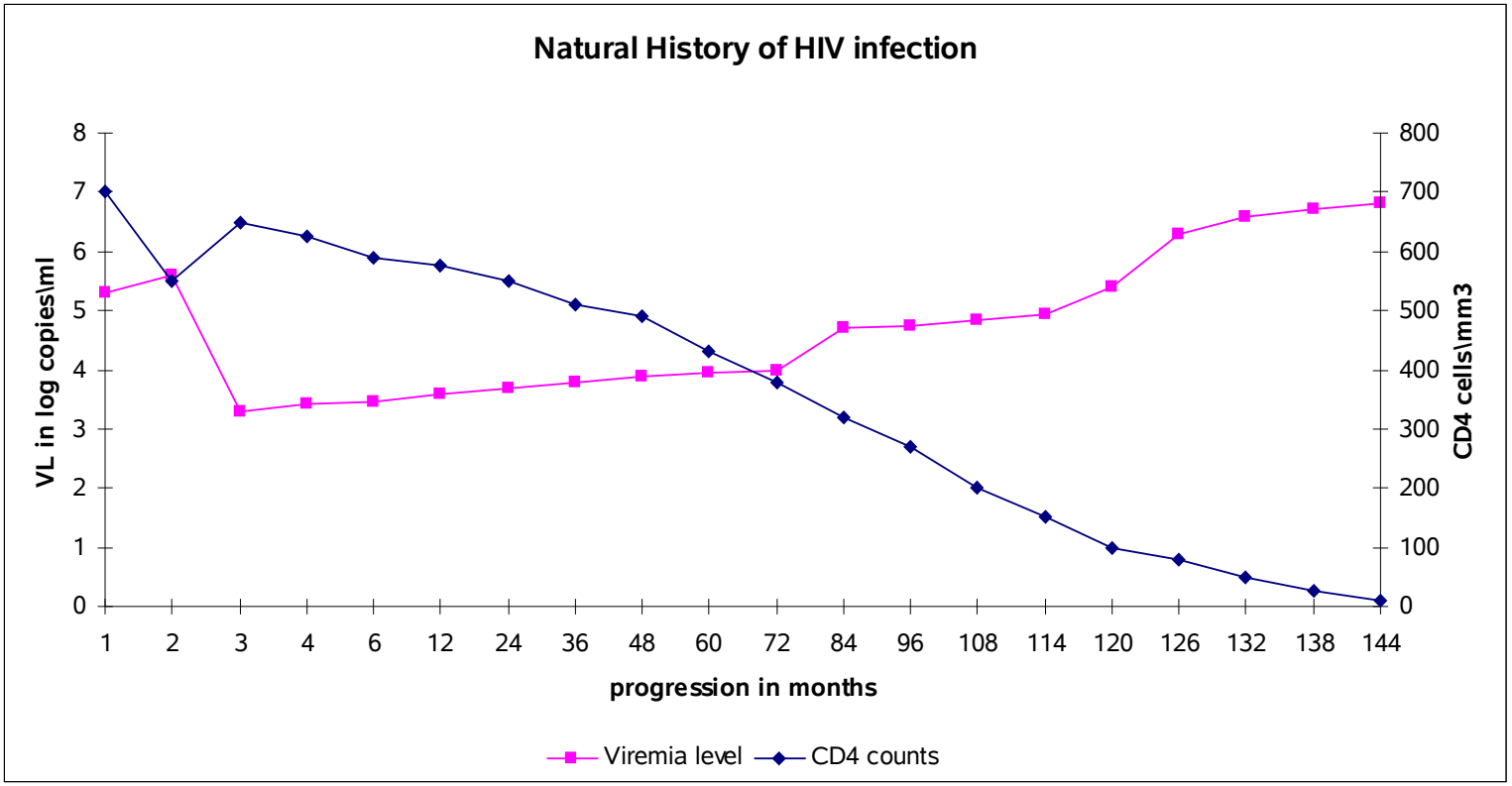
  Prediction, HGB based prediction, and TLC based prediction.

RESULTS

4.1 Literature data analysis

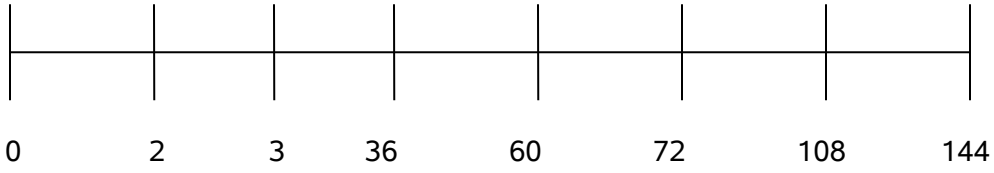
A. Natural History of HIV progression

FIG:7

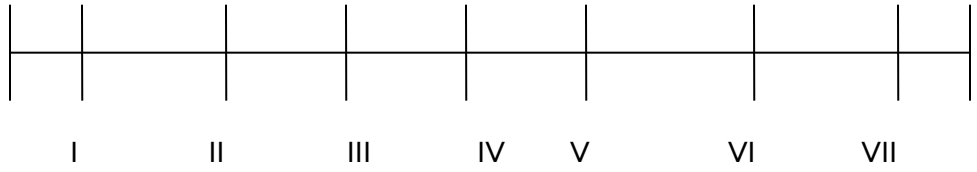

Natural History of HIV infection
VL in log copies\ml
CD4 cells\mm3
progression in months
0
1
2
3
4
5
6
7
8
100
200
300
400
500
600
700
800
1
2
3
4
6
12
24
36
48
60
72
84
96
108
114
120
126
132
138
144
Viremia level
CD4 counts

B. Categories in Natural History of HIV Progression

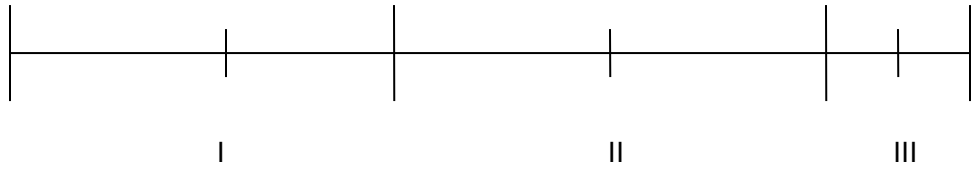
MONTHS



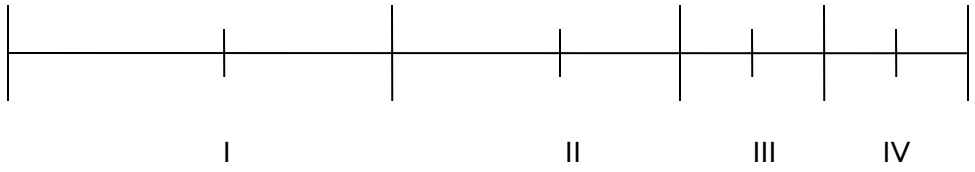
CATEGORY



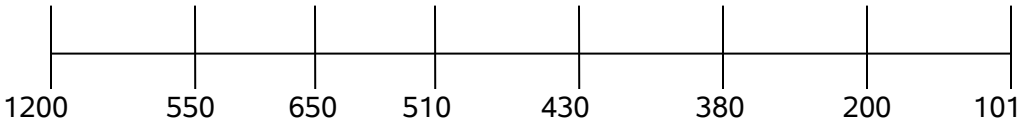
CDC



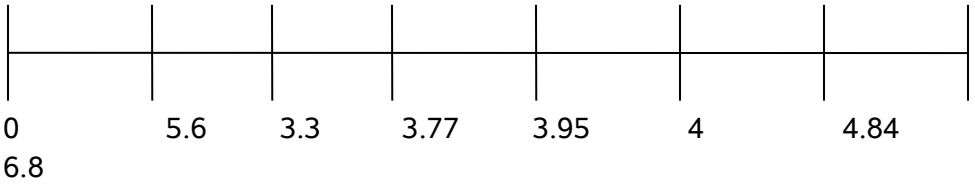
WHO



CD4



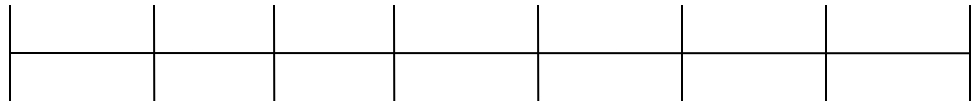
VL



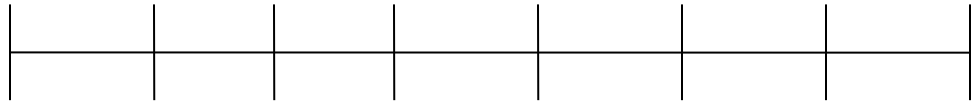
HGB

M/F

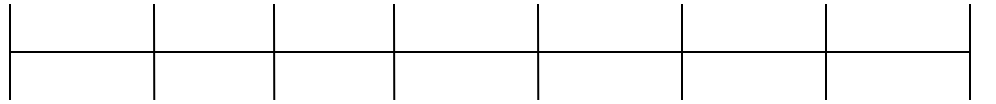
TLC



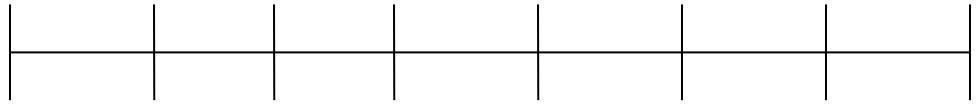
BMI



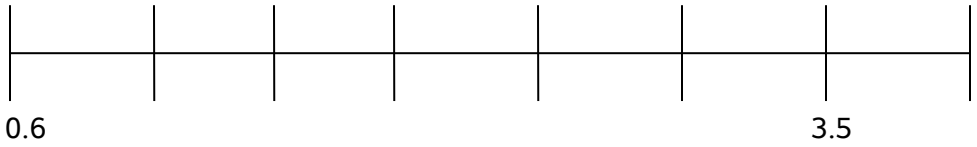
CD4%



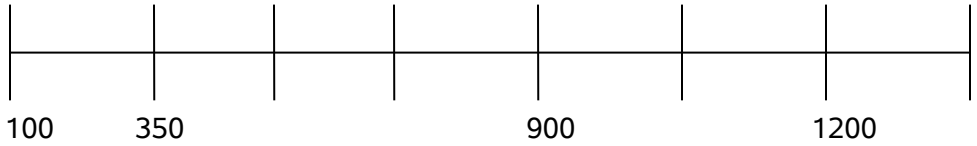
S neo



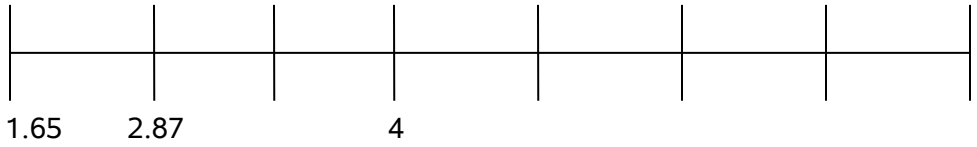
β_2M



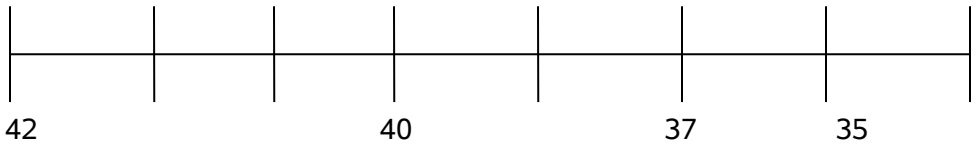
IL-2R



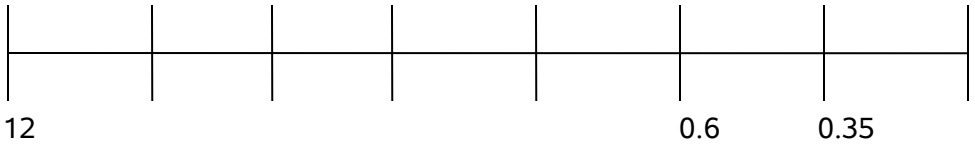
TNFα



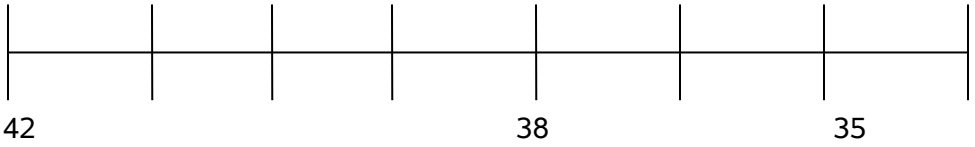
ALBUMIN



C-Rn



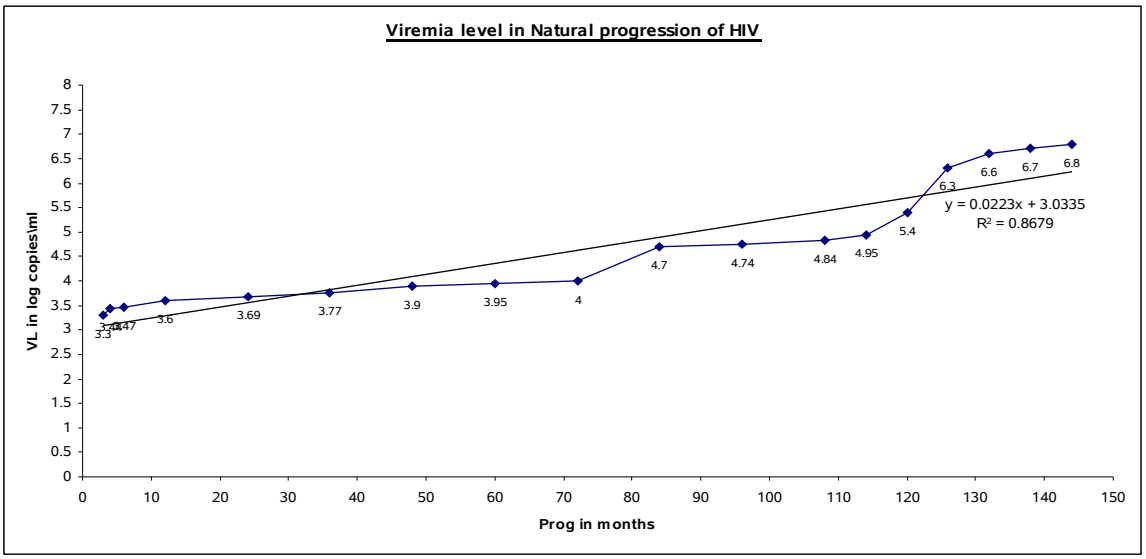
HGT



C. Changes in Markers trend in Entire progression of HIV

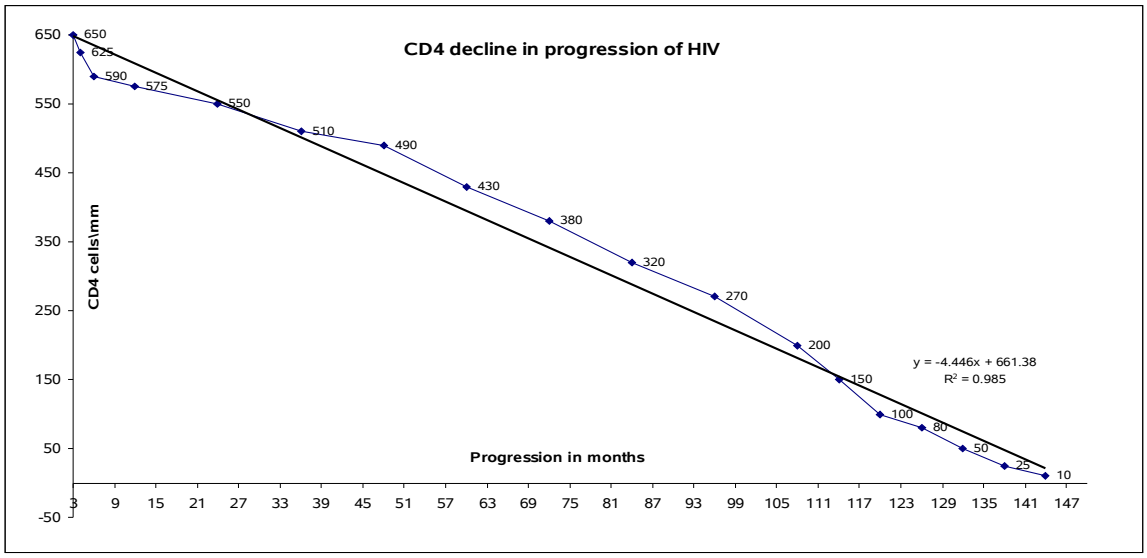
1. Trend of VL level in HIV progression

FIG:8



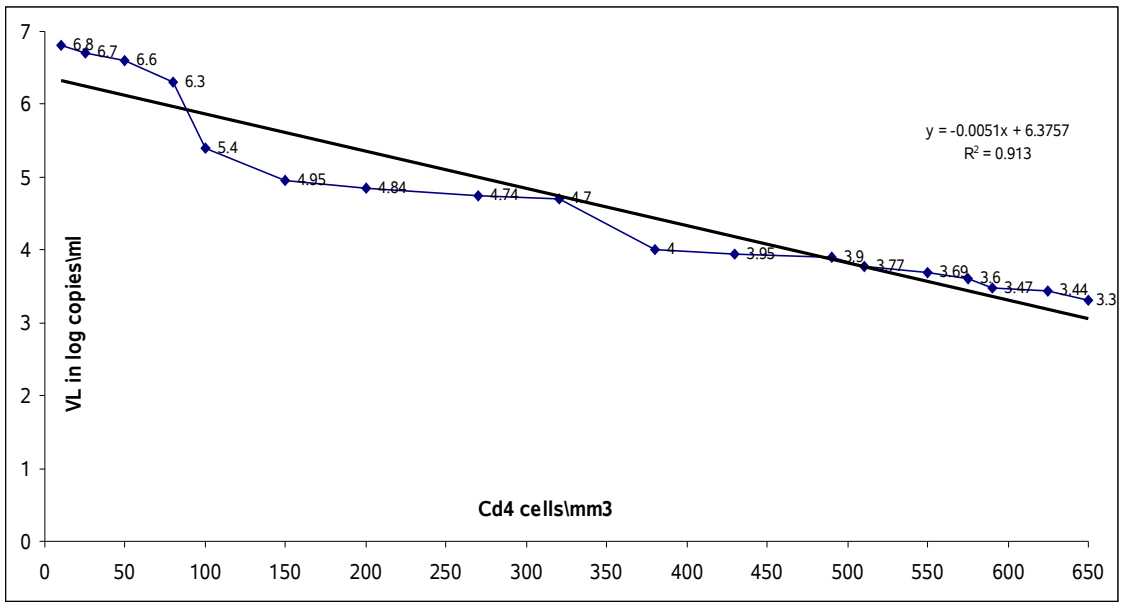
3. Trend of changes in CD4 count in HIV Progression

FIG:9



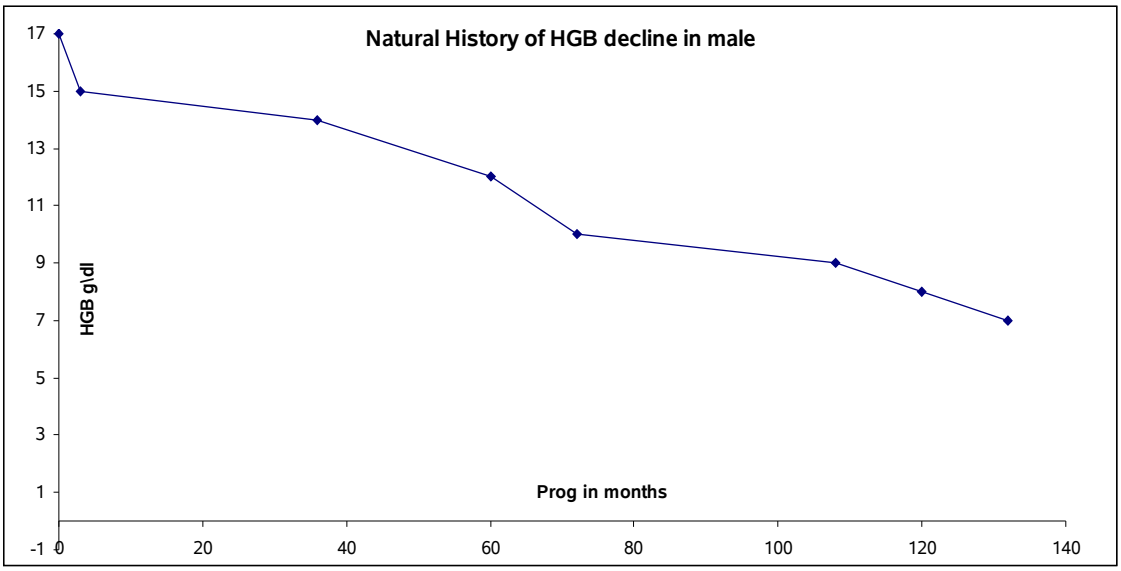
3. Correlation of CD4 and VL in HIV infection

FIG:10.



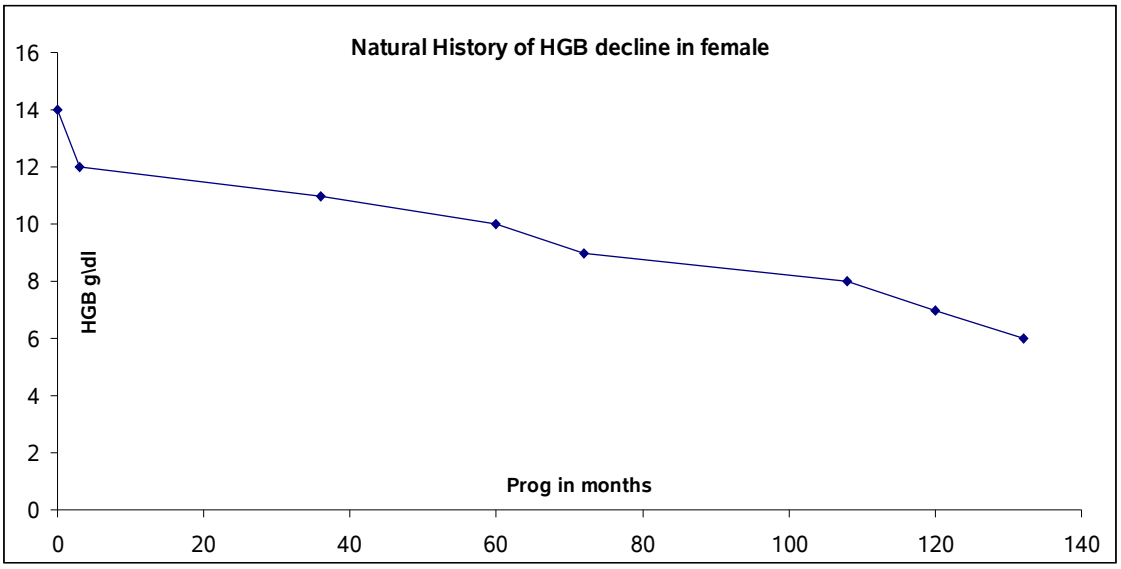
4. HGB decline in HIV progression in MALE

Fig:11



5. HGB decline in HIV progression in FEMALE

FIG:12

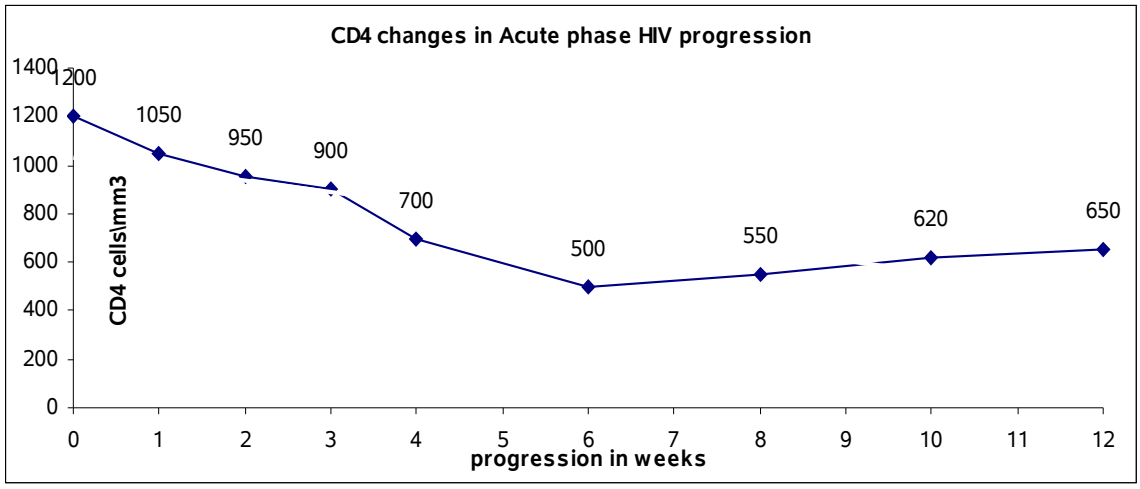


D. Changes of markers trend in different categories

1. Category I&II

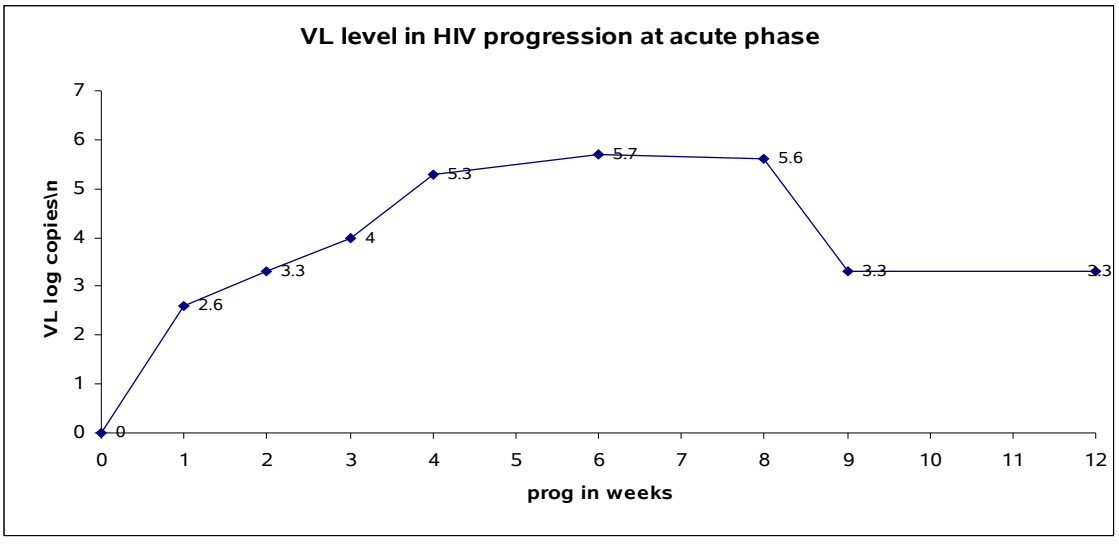
CD4 decline in category I&II

FIG:13



VL changes in Category I&II

FIG:14.



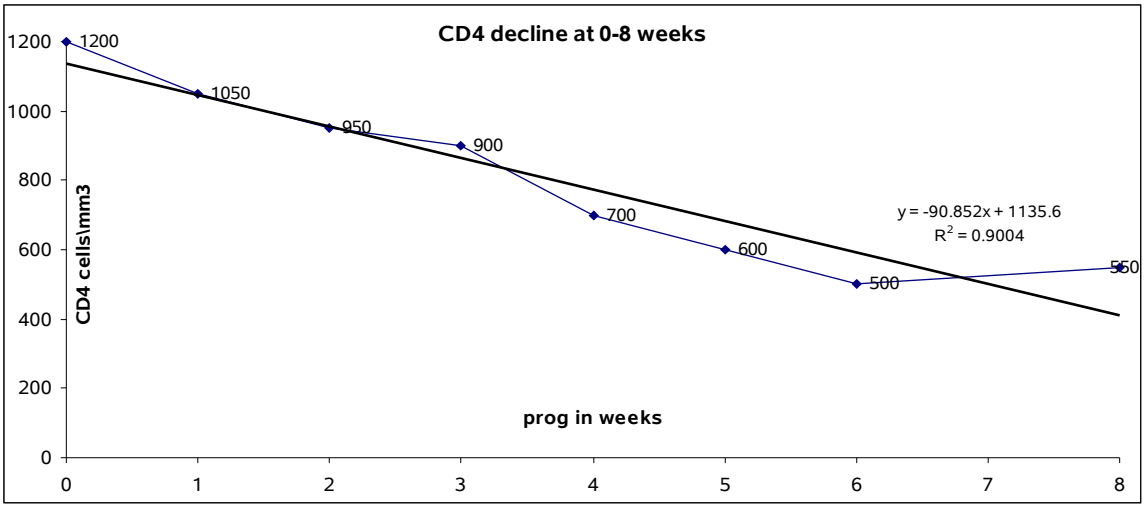
Data for Category I&II

Table :8

Progression in weeks	CD4 cells\mm3	VL in logcopies\ml	HGB g\dl M/F
0	1200	0	17/14
1	1050	2.6	
2	950	3.3	
3	900	4	
4	700	5.3	
6	500	5.7	
8	550	5.6	
9	600	3.3	
12	650	3.3	15/12

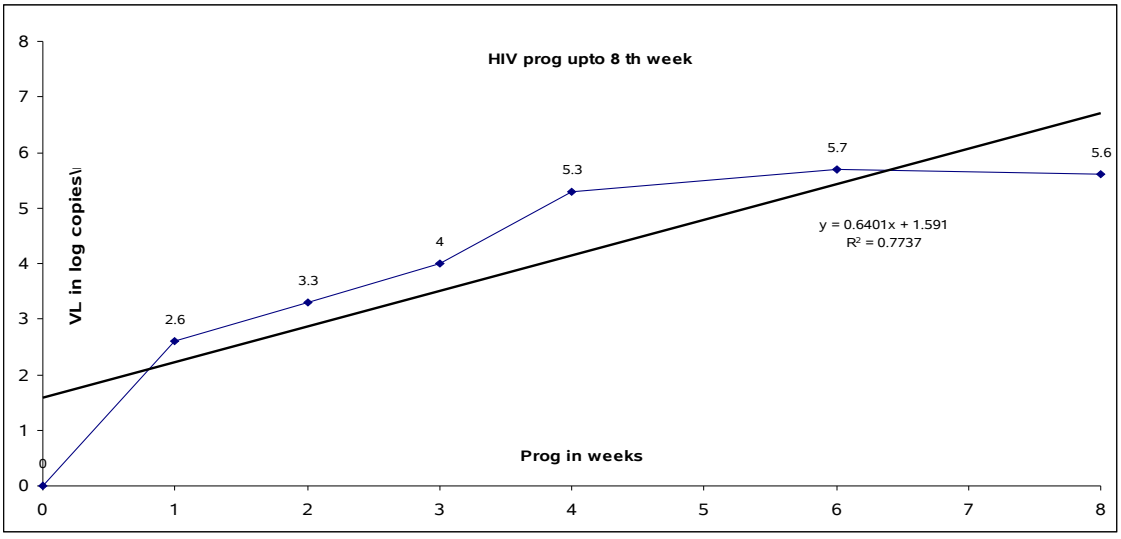
I. Change in CD4 count at 0-2 months

FIG:15.



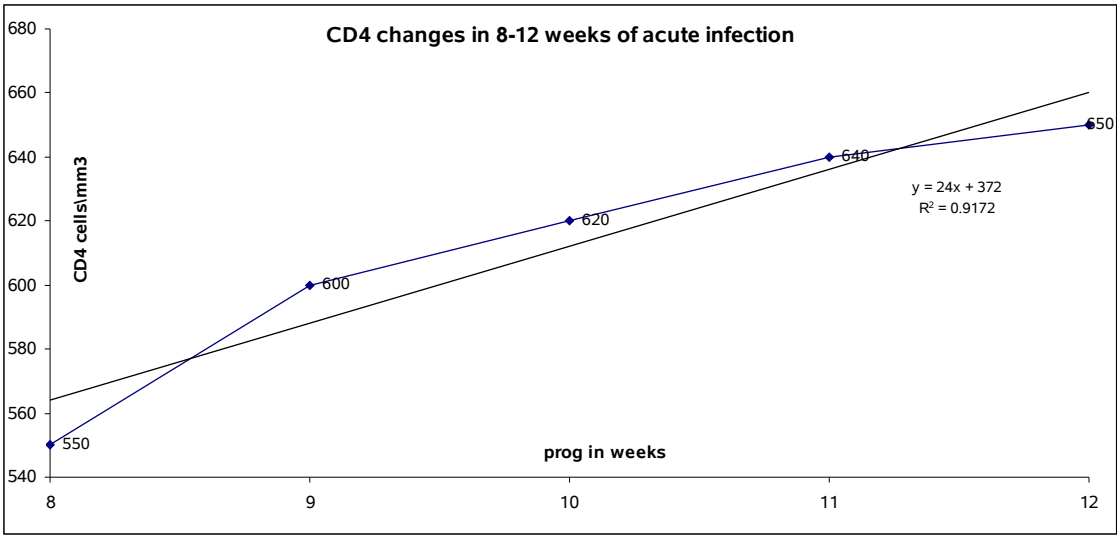
I. Change in VL level at 0-2 months

FIG:16.



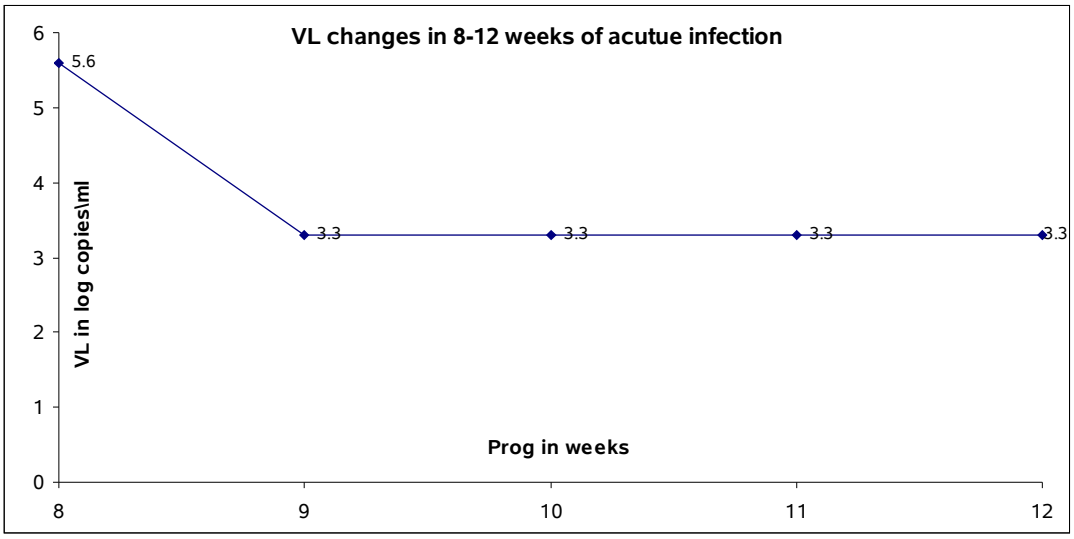
II. Change in CD4 count at 2-3 months

FIG: 17.



II. Change in VL level at 2-3 months

FIG:18.



2. Category III-VI

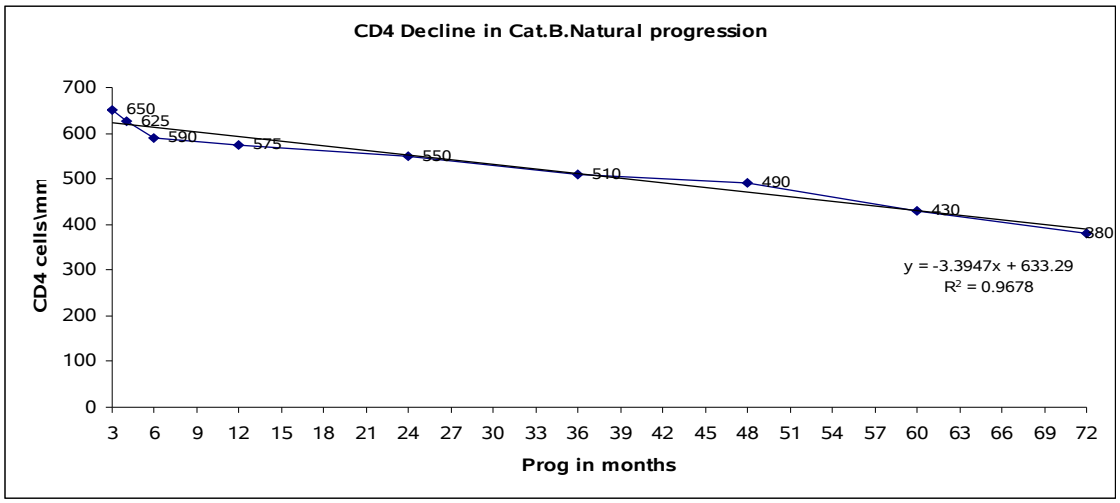
Data for category III-VI

TABLE:9

Progression in months	CD4 cells\mm3	VL log copies\ml	HGB g\dl
3	650	3.3	15\12
4	625	3.44	14\11
6	590	3.47	
12	575	3.60	
24	550	3.69	
36	510	3.77	12\10
48	490	3.90	
60	430	3.95	
72	380	4	10\9

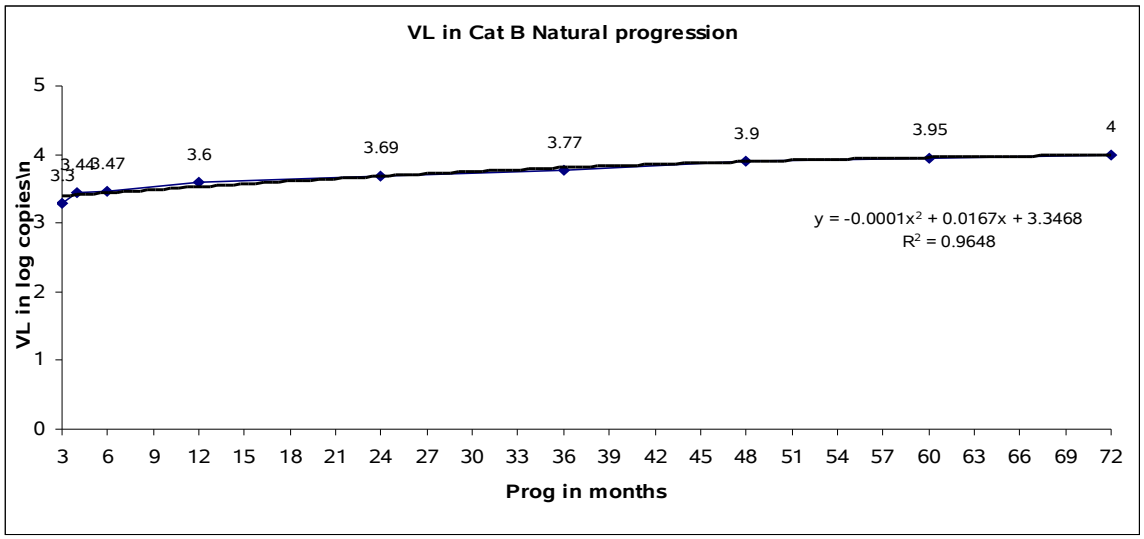
CD4 decline in category III-VI

FIG: 19.



VL changes in Category III-V

FIG :20.



3. Category VI-VII

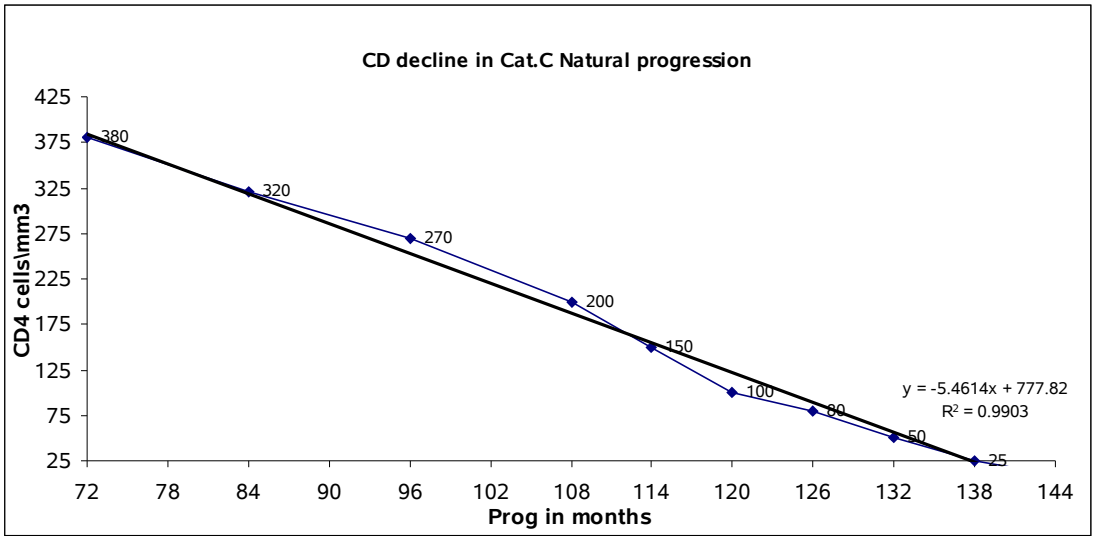
Data for Category VI-VII

TABLE: 10.

Progression in months	CD4 cells\mm3	VL log copies\ml	HGB g\dl
72	380	4	10\9
84	320	4.7	
96	270	4.74	
108	200	4.84	9\8
114	150	4.95	
120	100	5.4	7\6
126	80	6.3	
132	50	6.6	
138	25	6.7	
144	10	6.8	

CD4 decline in Category VI-VII

FIG :21



VL changes in VI-VII

FIG : 22.

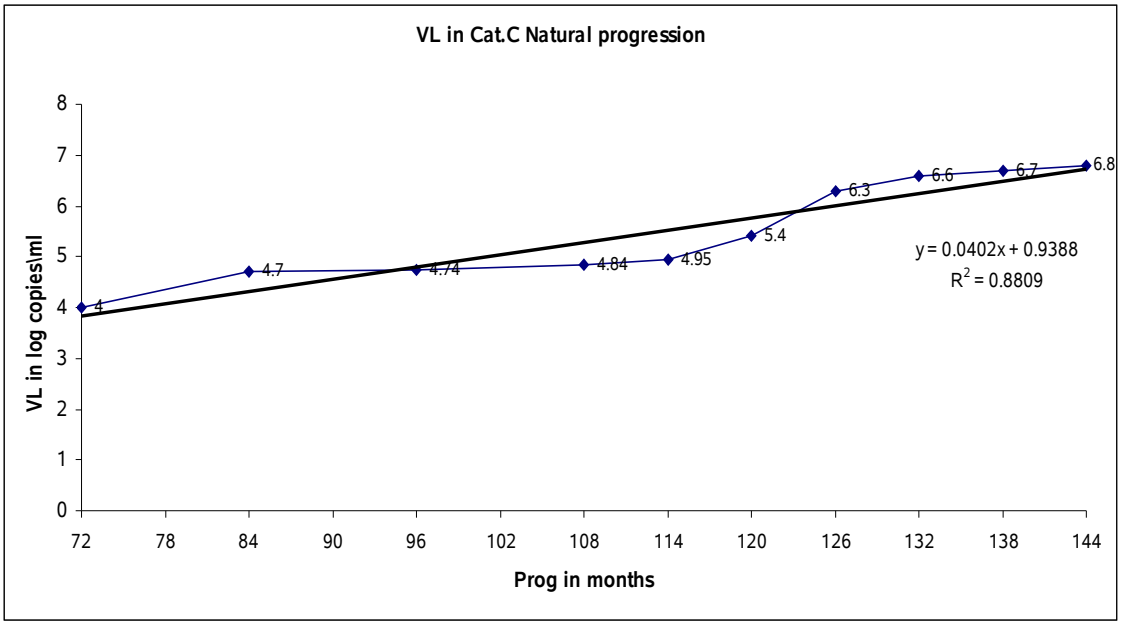


TABLE:11. F. Classification of HIV infection based on symptoms and changes in markers trend

Category	Months	Symptoms(HIV, OI, Drugs)	CD4 cells\ mm3	VL log copies\ ml	HGB g\dl F\M
I	2	• Fever • Mouth ulcer • Malaise • Arthralgia • Head ache • Loss of appetite • Rash • Pharyngitis • Anal ulcer • Genital ulcer • Weight loss	550	5.6	14\17

		• Diarrhoea • Thrush • Voimiting • Swollen lymph glands Drugs • GI distrubance			
II	3	• Aseptic meningitis • Peripheral neuropathy • Facial palsy • Guillian Barre synndrome • Branchial neuritis • Cognitive impairment	650	3.38	
III	36	• Mild fatigue • Low grade fever	510	3.77	11\14

		• Night sweats			
IV	60	• Cervical dysplasia patches • Persistant lymph adenopathy • Sore throat • Joint pain • Muscle pain • Cervical epithelial neoplasia OI • Severe diarrhea • Severe Weight loss o Drugs • GI intolerance • Colitis symptoms	430	3.95	10\12

V	72	• Recuurent respiratory tract infection OI • Purplish skin • Severe cough and weight loss • Sputum contain blood • Drugs • Skin eruption • Optic neuritis • Hematological symptoms • Anemia • Lymphophenia • Leucopenia • Bone marrow depression	380	4	9\10

		• Cardic complaints • Tingling, numbness Noticeable changes in the way of walk			
VI	108	• Dysplasia patches • Erythymatous Maculopapoular with lesions includind palms and soles OI • Fluffy white patch • Red inflamed skin • Severe irritation • Leisons on genitals • Patches on armbits • Bleeding from mucous membrane • Shingles (painful blister in a band of	200	4.84	8\9

		red skin) • Thrush (thick white coating) • Tingling,numbness • Neurological dysfunction • Pure RBC aplasia • Neutropenia • Anemia • Thrombocytopenia • More than I month chronic ulcer • Bronchitis • Fungal nail infection • Dermatitis Drugs • GI intolerance			

VII	144	• HIV • Primary CNS syndrome • Severe watery diarrhea • Severe Weight loss • Gastro enteritis OI • Pseudo membraneous plaques • Pain on swallowing • 2-3 weeks head ache • Phtophobia • Neurological dysfunction • Pure RBC aplasia	10	6.7	6\7

		• Anemia • 2-3 week dry cough • fever more than 1 months • Disproportionate breathlessness • Sore throat • Sneezing • Oesophagal symptoms • Reduced visualactivity hemorrohogic exudates along retinal vessels • Loss of memory and depression • Paralysis • Leisons in cerebraum			

		• Unconsiousness • More than 1 month chronic GI problem • Chronic blood loss • Anemia • DRUGS • Liver function impaired • Hypo kalemia • Bone marrow depression • Weight loss • GI intolerance • Lactic acidosis • Abdominal pain • Glycosuria • Proteinmia • Leucopenia • Blister formation			

		• Skin rash • Allergy • Skin eruption • Anorexia • Thrombocytopenia • Anemia(drug d) • Hyperglycemia • Hyper lipidemia • Poly urea • Poly pagia			

4.2. Existing Data Analysis

Clinical and laboratory profile of study group

Case No	Class	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	age	sex	Hb	TLC	CD4 cells
9	I						y10		y10									y										41	m	12.7	2244	
8	I												y										y					28	m	17.1	2170	
22	I							y	y5								Y				y							46	m	15.3	2100	
2	I			y		y			y			y																25	m	10.6	2000	
25	I				y10				y10									y				y						59	m	9.1	1854	
36	I								y			y	y								y							30	f	12.4	1800	
30	I	y				y	Y			y	y		y															61	m	13	1734	
32	III	y			y									y	y						y				y		y	49	m	10.7	1722	
49	III	y15							y15									y	y			y			y			38	m	9.6	1700	380

18	III	y5							y5				y15								y							47	m	14	1683	
12	III								y5								Y			y								29	m	13	1640	
26	IV						Y													y		y	y					49	m	11.7	1620	
23	IV				y																y	y	y	y				y	63	m	11	1564	
43	IV										y		y								y								42	m	11.5	1540	
19	IV				y10		Y		y30					y	y										y				33	m	11	1536	
14	IV						y15							y	y15			y			y	y10	y						33	m	10.1	1518	
15	IV							y30	y30										y										42	m	12	1500	
48	IV	y					Y		y		y		y			y					y		y						75	m	10	1500	

24	V	y10				y		y			y							y										29	f	9.4	1482	410
38	V								y					y	y						y							50	m	10.7	1480	
44	V				y5									y	y						y				y			38	m	10.1	1474	
41	V	y15							y15	y				y30	y			y				y						47	m	9.3	1458	
35	V						Y							y	y				y		y	y			y			32	m	10.1	1456	310
31	V				y10		Y		y10	y																		38	m	14.2	1452	
33	V				y		Y		y5				y								y	y						28	m	11.4	1450	440
40	V	y30			y15				y					y30												y5		33	m	11.1	1450	
20	V				y15		y15		y15					y	y						y					y10	y	27	m	11.3	1407	
16	V							y15	y10				y15													y5		39	m	11.6	1400	
27	V	y15			y					y											y	y		y				48	f	9	1400	324
34	VI				y				y		y		y				Y											38	m	12.	138	

																													8	6	
1	VI		y		y10	y	Y		y30					y	y	y		y			y							39	m	8	1375	
50	VI							y	y10				y	y	y					y							33	m	12	1365	
10	VI	y10			y15			y													y	y		y				35	m	10.8	1320	
28	VI										y10		y15								y		y10					34	m	12.8	1276	
17	VI						Y		y15		y										y			y				48	m	9	1250	
47	VI											y10						y			y							57	f	10.6	1125	
46	VI			y30			y30											y								y15		31	f	8.2	1092	205
45	VI			y					y30			y	y	y15	y15						y					y15		24	m	8	1090	190
5	VI				y				y30					y10	y10						y							39	m	10.5	1080	
4	VI								y15								y15	y										47	m	12	1024	

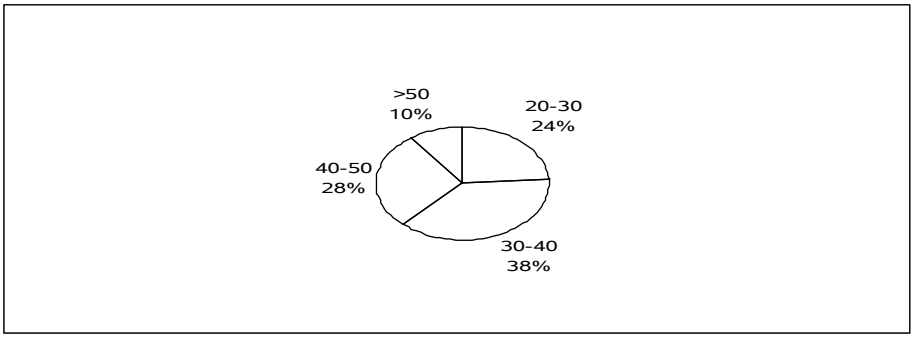
42	VII	y30		y	y				y30									y				y15	y			y30		44	m	7.6	950	84
29	VII			y			Y		y10									y										36	m	8.4	935	225
3	VII			y	y				y						y													26	m	10.6	900	
37	VII	y10		y	y15		y15		y30		y			y	y			y			y			y		y15	y	52	m	7.2	896	185
7	VII				y				y					y	y						y							30	m	9	880	
13	VII	y30			y15				y15	y				y	y15			y			y						y	32	m	8.4	810	78
11	VII				y15	y			y	y				y10	y10						y							25	m	9.3	793	102
39	VII								y30	y															y	y		25	m	8.2	792	178
6	VII								y10					y	y					y					y	y		46	m	7.2	510	
21	VII	y	y				Y		y30										y		y							40	m	7.6	390	108
I	7	1	0	1	1	2	2	1	5	1	1	2	3	0	0	0	1	2	0	0	2	1	1	0	0	0	0					0
III	4	3	0	0	1	0	0	0	3	0	0	0	1	1	1	0	1	1	1	1	2	1	0	0	2	0	1					1
IV	7	1	0	0	2	0	4	1	3	0	2	0	2	2	2	1	0	2	1	1	5	3	3	1	0	0	1					0
V	11	4	0	0	6	1	4	2	7	3	1	0	2	6	5	0	0	2	1	0	6	4	0	1	2	3	1					4
VI	11	1	1	2	4	1	3	2	7	0	3	2	4	4	4	1	2	4	0	1	7	1	1	2	0	2	0					2
VII	10	4	1	4	6	1	3	0	10	3	1	0	0	5	6	0	0	4	1	1	5	1	1	1	2	4	2					7
Sy	50	14	2	7	20	5	16	6	35	7	8	4	12	18	18	2	4	15	4	4	27	11	6	5	6	9	5					14

m																														

A. Characteristics of subjects (N=50)

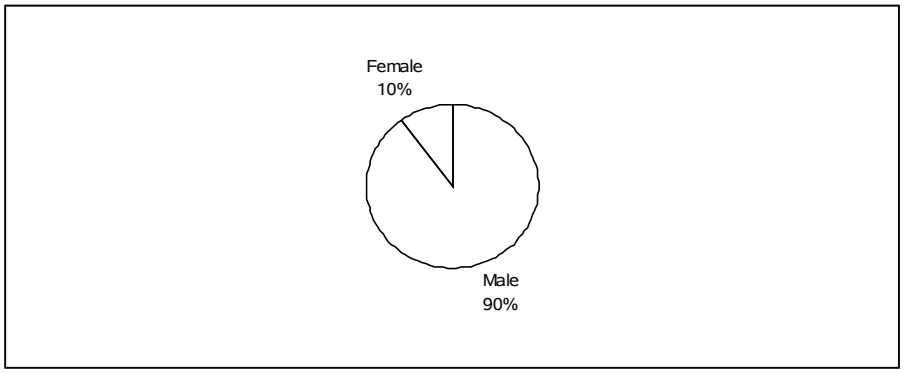
1. Age group in study population

FIG:23.



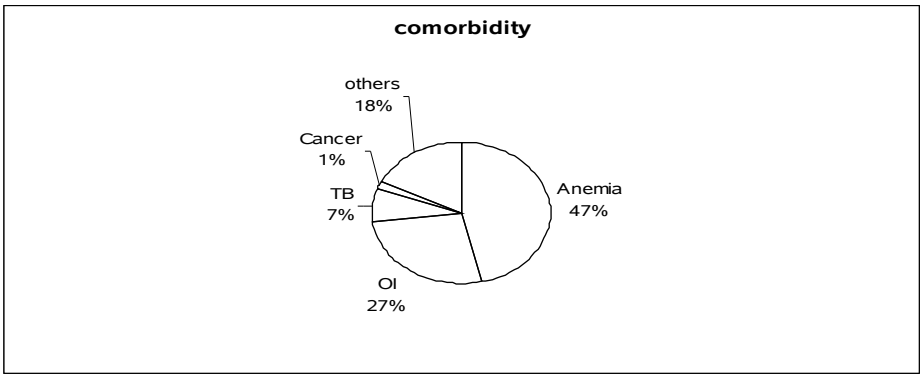
2. Percentage of male and female participants

FIG: 24.



3. Comorbidity in study population

FIG:25.



B. I. Clinical symptoms frequently observed in total study population

1. Abdominal pain*
2. Acute pancreatitis
3. Anemia*
4. Cough with expectoration
5. Diabetes melitis
6. Difficulty in breathing
7. Enlarge lymph node*
8. Fever*
9. Gastritis*
10. Guiddiness*
11. Head ache*

12. Joint pain*
13. Loss of appetite*
14. Loss of weight *
15. Multiple Liver abscess
16. Night sweating*
17. OI symptoms
18. Oral candidiasis
19. Oral ulcer*
20. Others
21. Respiratory tract infection *
22. Slurring of speech*
23. TB
24. Vomiting *
25. Watery diarrhoea*
26. White mucoid sputum

Note : * indicates the HIV symptoms observed in study group

Others

1. Acute Coronary syndrome
2. Bronchitis
3. Chest pain
4. Chronic ulcer
5. Decrease in urine output
6. Edema
7. Focal fitness
8. General weakness
9. Hemorrhoids
10. Ischemic Heart Disease
11. Numbness
12. Pain on swallowing
13. Pleural effusion
14. Sneezing
15. Spasm

Symptoms observed in study group

FIG:26.

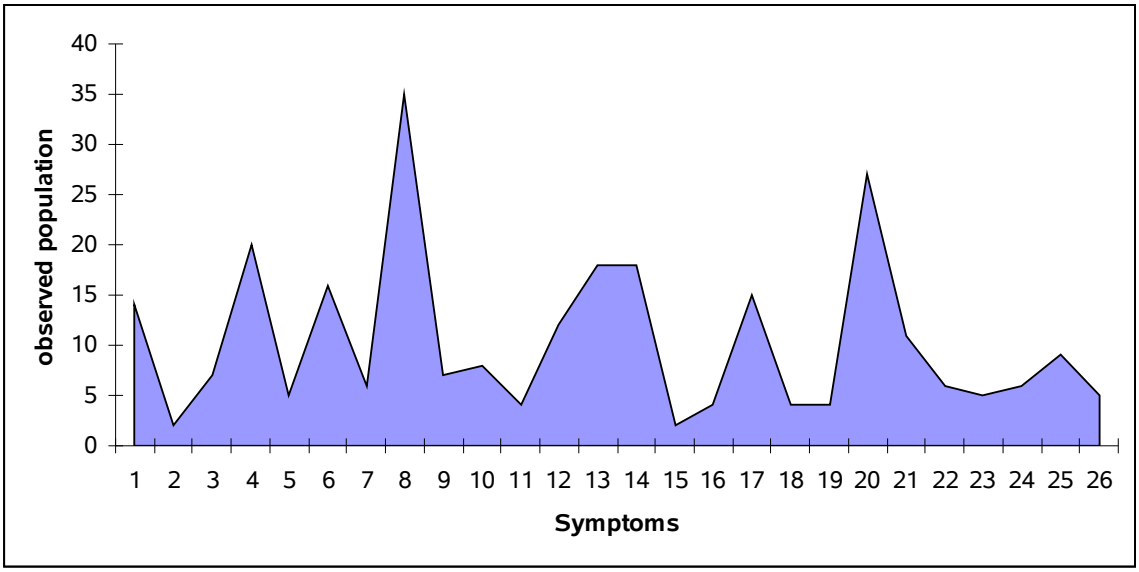


Fig 27 Symptoms distribution

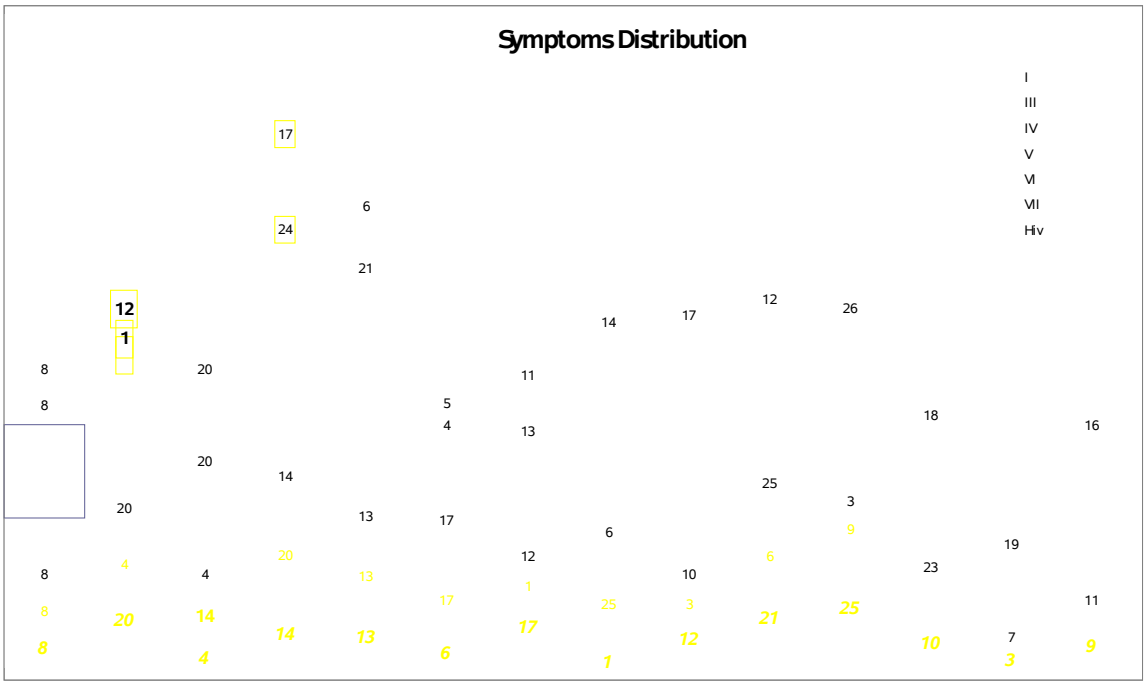
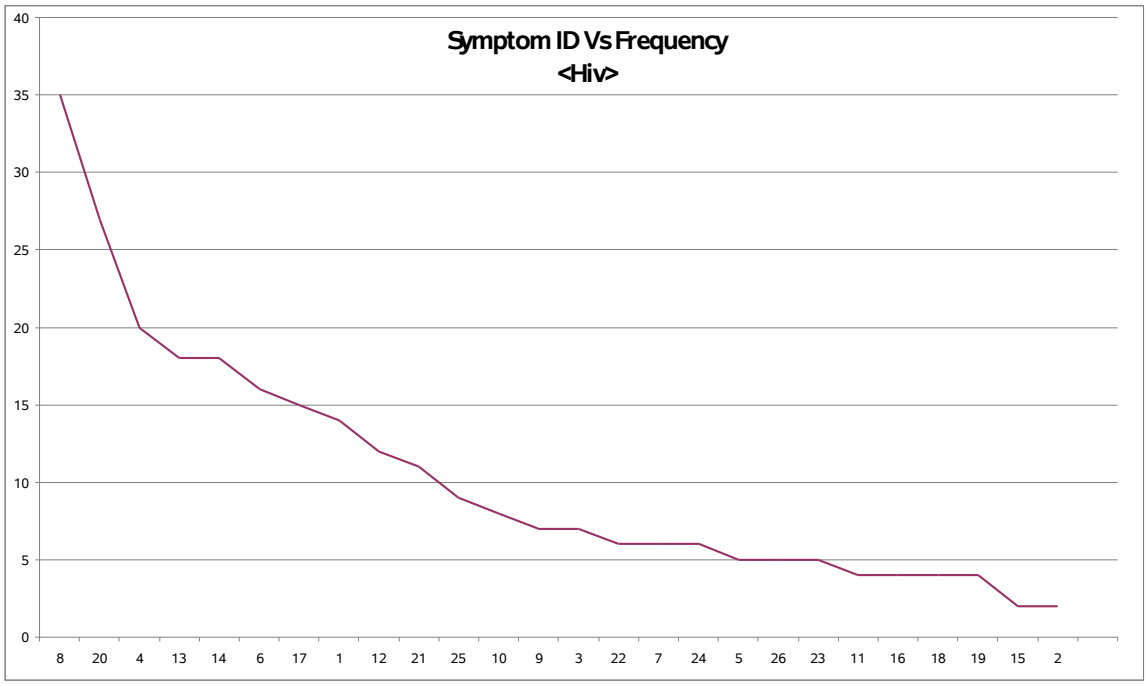


Table 12 frequently distributed symptoms

Sym ID	I	III	IV	V	VI	VII	Sym
8	5	3	3	7	7	10	35
20	2	2	5	6	7	5	27
4	1	1	2	6	4	6	20
13	0	1	2	6	4	5	18
14	0	1	2	5	4	6	18
6	2	0	4	4	3	3	16
17	2	1	2	2	4	4	15
1	1	3	1	4	1	4	14
12	3	1	2	2	4	0	12
21	1	1	3	4	1	1	11

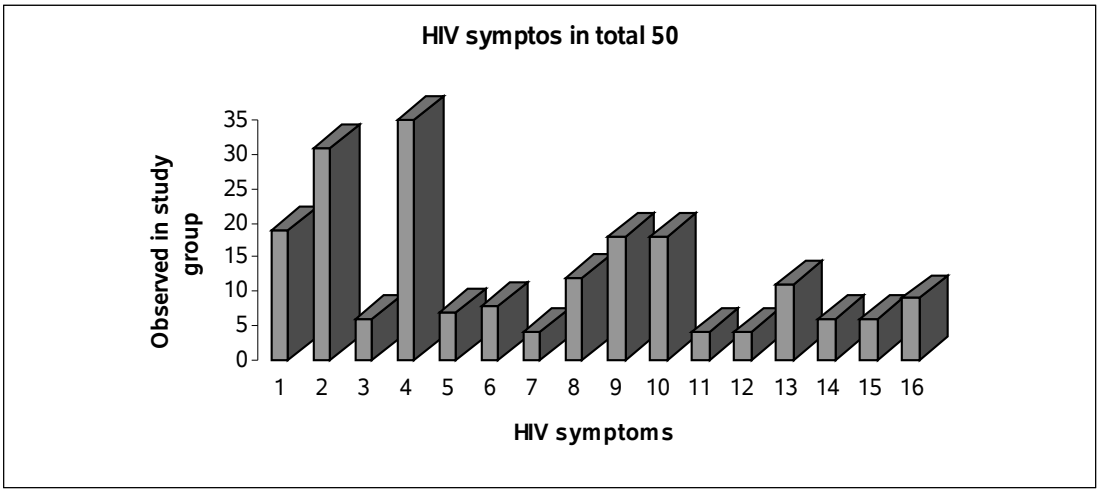
25	0	0	0	3	2	4	9
10	1	0	2	1	3	1	8
9	1	0	0	3	0	3	7
3	1	0	0	0	2	4	7
22	1	0	3	0	1	1	6
7	1	0	1	2	2	0	6
24	0	2	0	2	0	2	6
5	2	0	0	1	1	1	5
26	0	1	1	1	0	2	5
23	0	0	1	1	2	1	5
11	2	0	0	0	2	0	4
16	1	1	0	0	2	0	4
18	0	1	1	1	0	1	4
19	0	1	1	0	1	1	4
15	0	0	1	0	1	0	2
2	0	0	0	0	1	1	2

Fig 28



HIV symptoms in total study group

FIG:29



B. II.HIV symptoms in different study group

TABLE: 13

Category	Total symptoms Frequently observed	HIV symptoms
I	15	11
II	0	0
III	7	3
IV	14	12
V	20	11
VI	16	10
VII	21	12

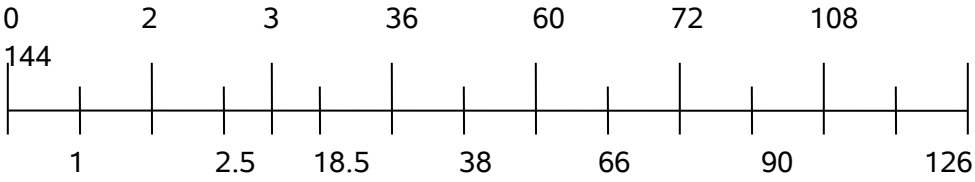
Table 14

Unique symptom of HIV in entire population

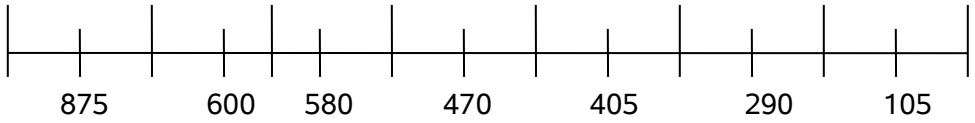
Hiv	VII	VI	V	IV	III	I
8	8	8	8	20	8	8
20	4	20	20	6	1	12
4	14	4	4	8	20	20
14	20	14	13	21	24	17
13	13	13	14	22	21	6
6	17	17	6	4	4	5
17	1	12	1	13	13	11
1	25	6	21	14	14	
12	3	10	25	17	17	
21	6	25	9	12	12	
25	9	3	17	10	26	
10		23	12		18	
3		7	7		19	
9		11	24		16	

C. Laboratory parameter analysis

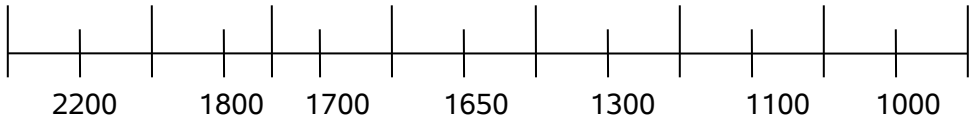
Mid month



Mid CD4

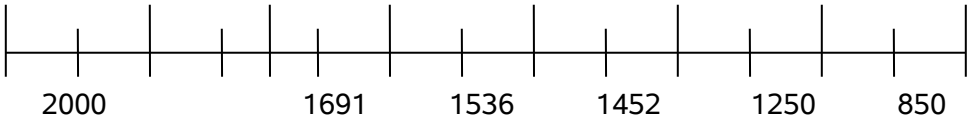


Mid TLC

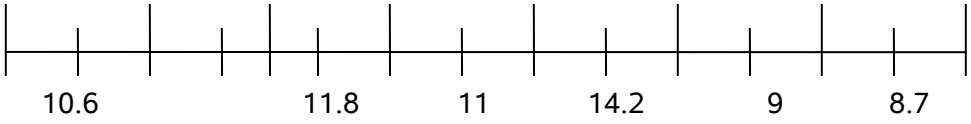


Mid HGB

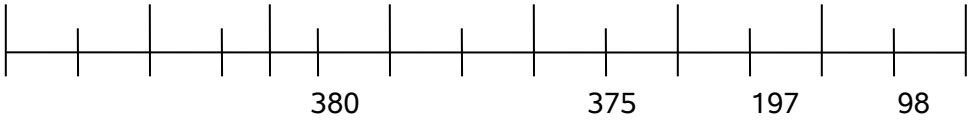
TLC Median (from study)



HGB Median (from study)



CD4 Median (from study)



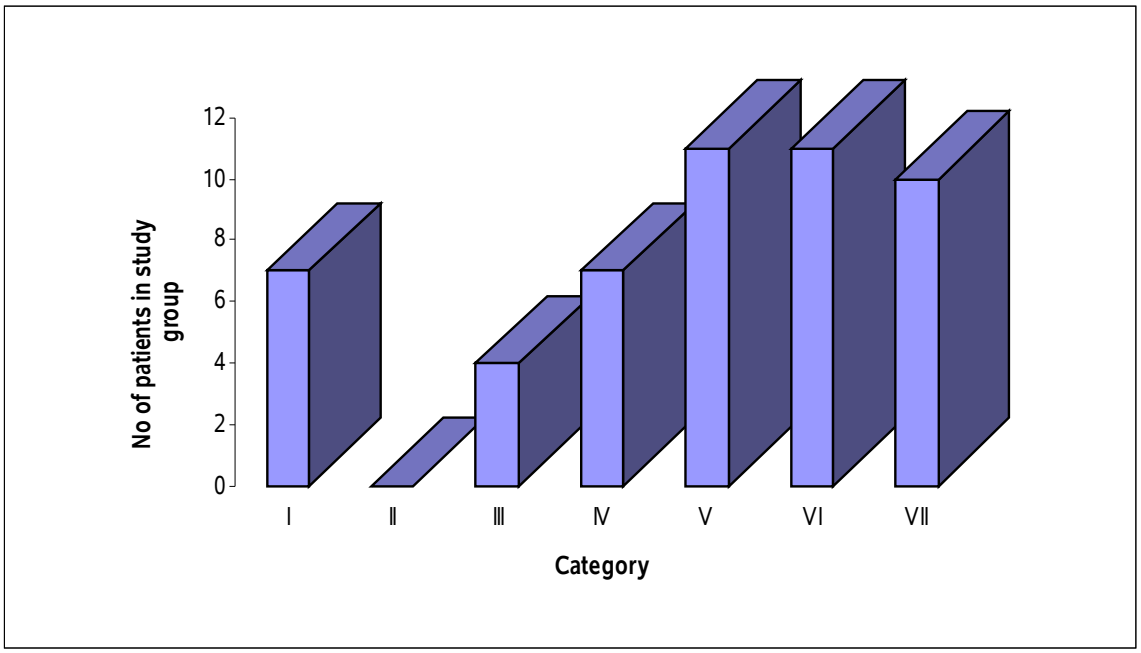
D. Predicted category of Study group

I. Population of HIV in different Category

TABLE:15

Category	Study no
I	7
II	0
III	4
IV	7
V	11
VI	11
VII	10

FIG:30



Anemia condition in Study group

TABLE : 16

Category	Study no
I	2
II	0
III	2
IV	4
V	6
VI	7
VII	10

FIG:31.

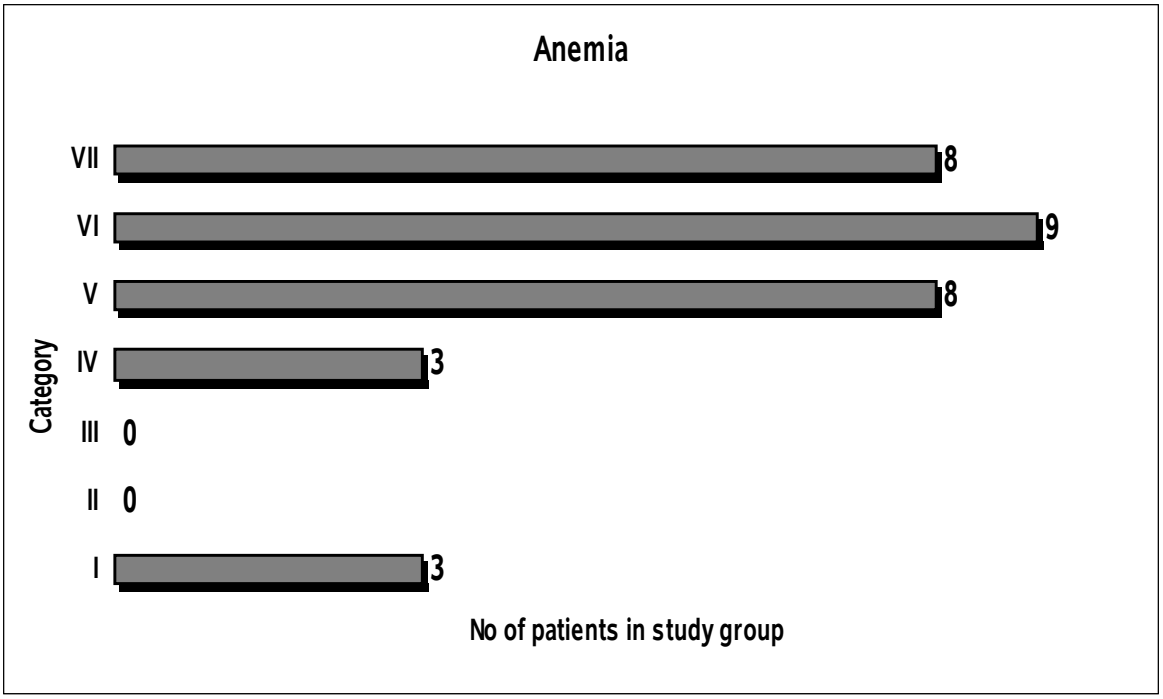

Anemia
VII
VI
V
IV
III
II
I
8
9
8
3
0
0
3
Category
No of patients in study group

Fig 32

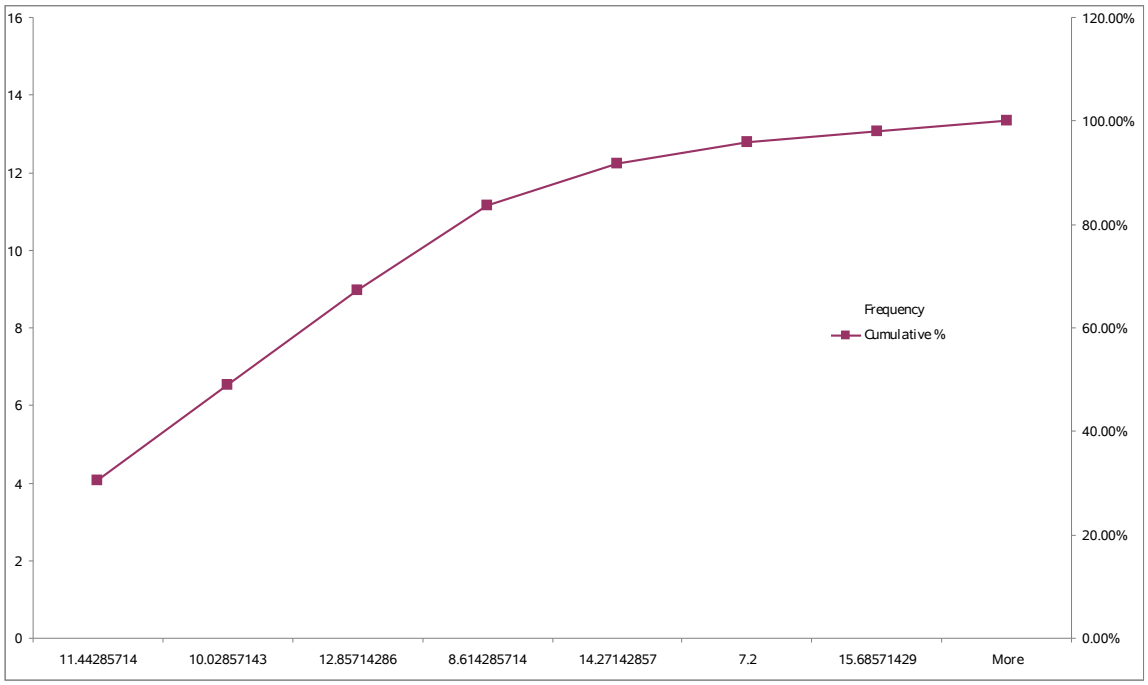


Fig 33

TLC count in Study group

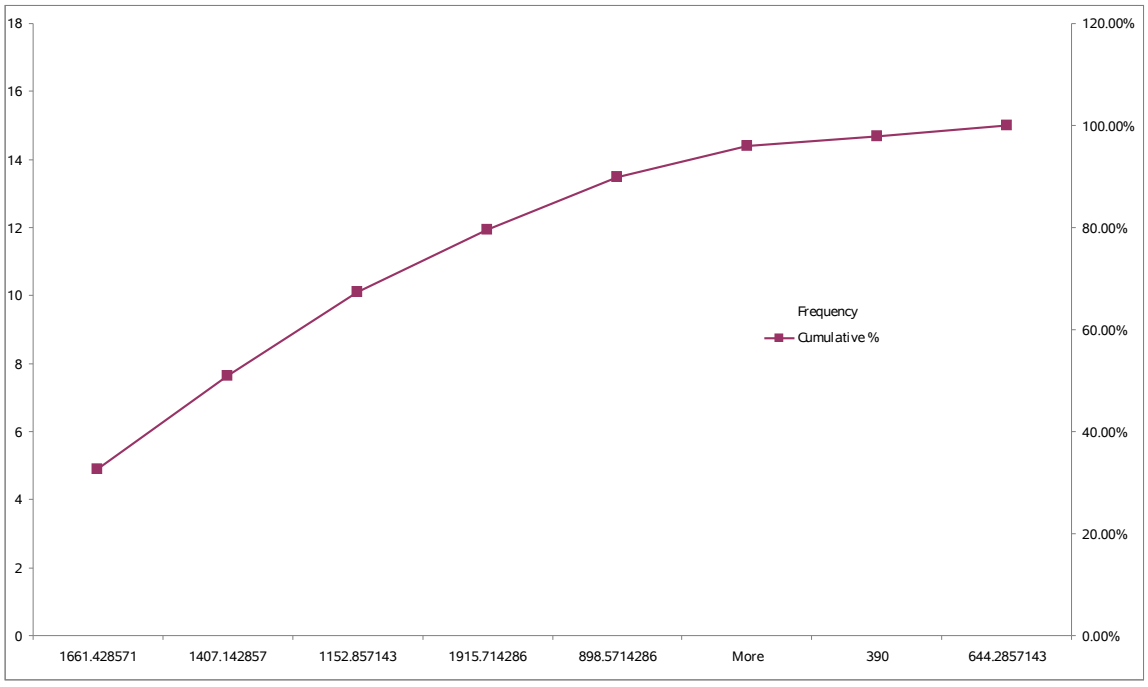


Fig 34

Age group in study population

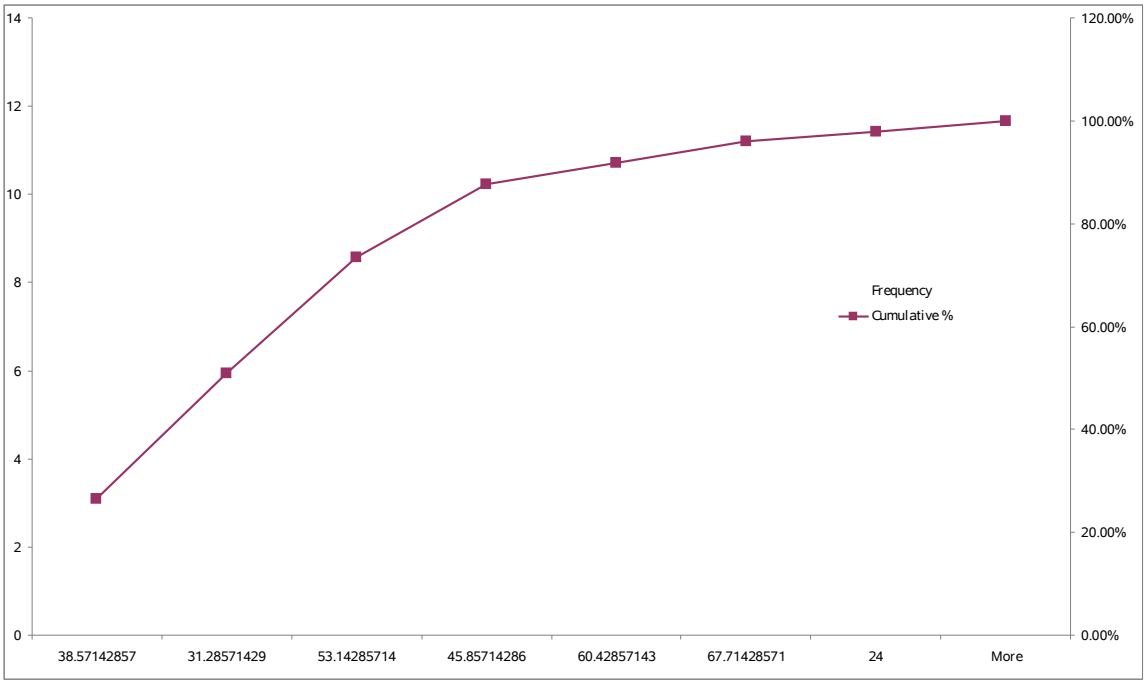
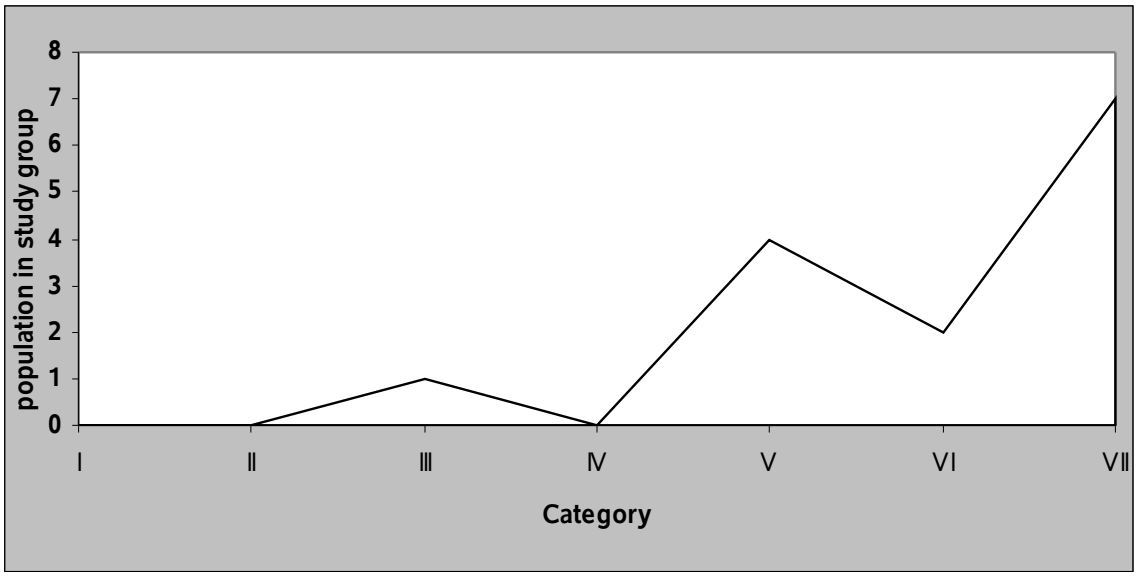


TABLE: 17.II. Predicted category of CD4 count measured patients

Category	Study no
I	0
II	0
III	1
IV	0

V	4
VI	2
VII	7

FIG:35



D. Prediction of CD4

TLC,HGB,AGE of 14 CD4 category

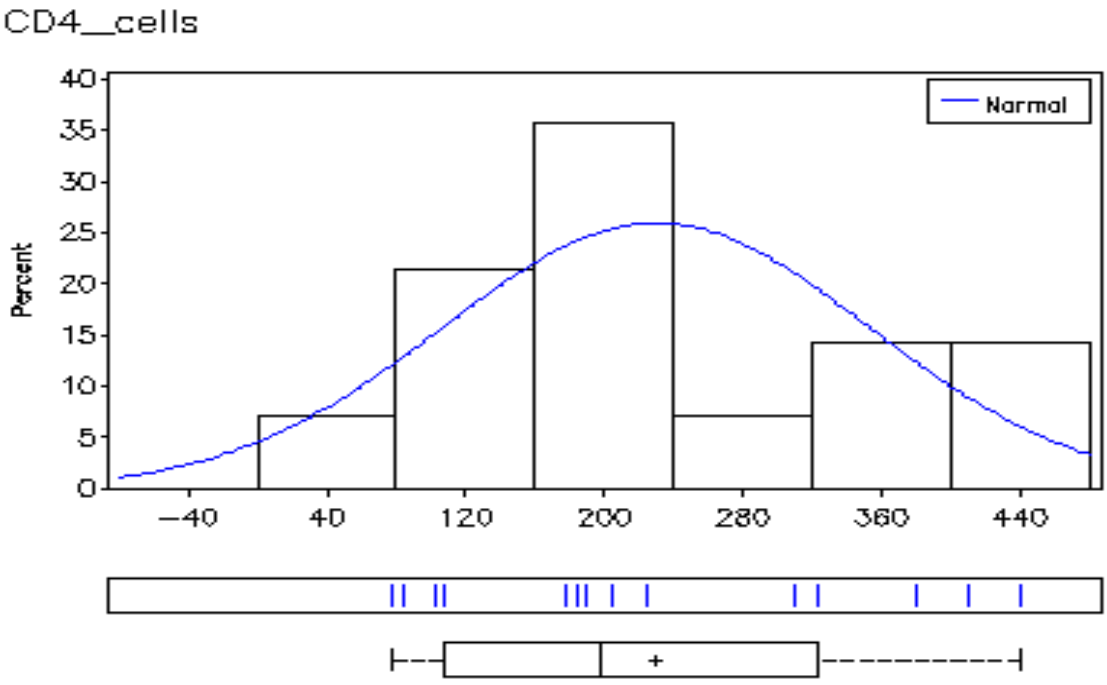
Table 18

Case No	Class	age	sex	HB	TLC	CD4 cells
33	V	28	m	11.4	1450	440
35	V	32	m	10.1	1456	310

49	III	38	m	9.6	1700	380
24	V	29	f	9.4	1482	410
11	VII	25	m	9.3	793	102
27	V	48	f	9.0	1400	324
29	VII	36	m	8.4	935	225
13	VII	32	m	8.4	810	78
46	VI	31	f	8.2	1092	205
39	VII	25	m	8.2	792	178
45	VI	24	m	8.0	1090	190
21	VII	40	m	7.6	390	108
42	VII	44	m	7.6	950	84
37	VII	52	m	7.2	896	185

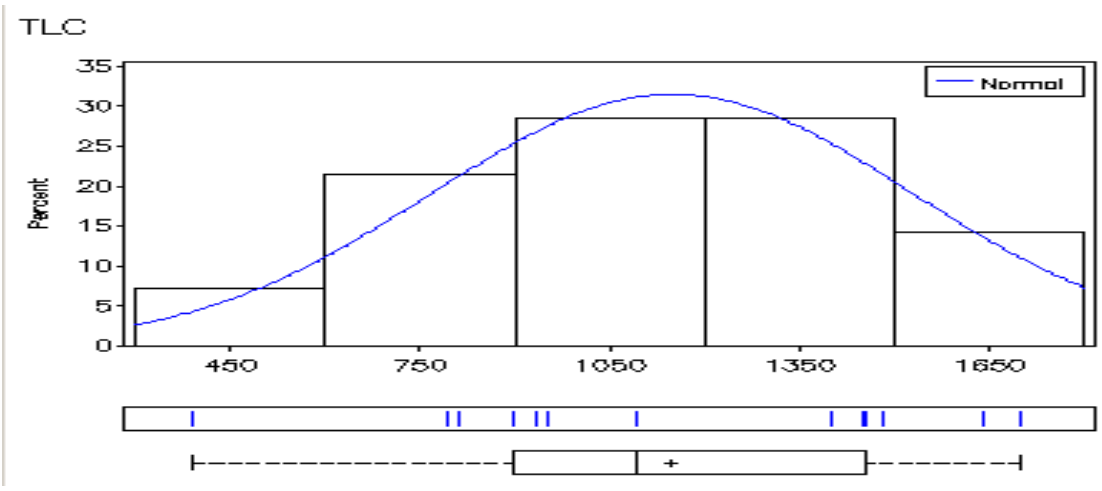
Observed CD4 counts in Study population

Fig 36



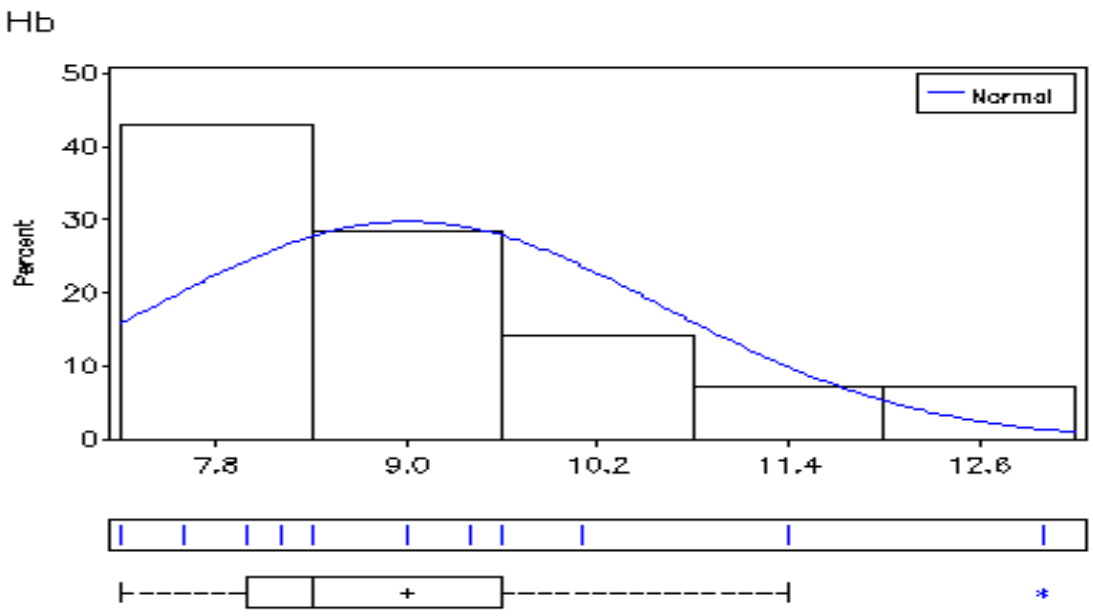
TLC counts in CD4 count measured patients

Fig 37



HGB level in CD4 count measured patient

Fig 38



Regression analysis of 3 predictors of HIV infection

Predicded values of entire study population

Table 19

446	337	426	0.9580	1470	1.0569
283	346	336	0.9612	1215	0.9731
300	411	419	0.9830	1240	0.9823
251	322	293	0.9530	1164	0.9542
340	338	364	0.9586	1304	1.0043
202	184	166	0.9065	1087	0.9243
186	231	197	0.9222	1061	0.9138
170	230	194	0.9220	1036	0.9031
105	173	163	0.9026	934	0.8573
186	142	139	0.8922	1061	0.9138
137	23	93	0.8520	985	0.8808
275	142	123	0.8923	1202	0.9685
137	189	169	0.9080	985	0.8808
202	147	140	0.8940	1087	0.9243
552	572	996	1.0374	1637	1.1038
910	550	1480	1.0300	2199	1.2330
763	529	1200	1.0230	1969	1.1847
381	499	623	1.0130	1368	1.0253

259	456	426	0.9984	1176	0.9590
527	440	688	0.9930	1598	1.0934
576	421	695	0.9864	1675	1.1139
389	417	511	0.9852	1381	1.0294
657	406	734	0.9813	1803	1.1461
576	393	633	0.9770	1675	1.1139
470	387	533	0.9750	1509	1.0682
414	370	460	0.9694	1419	1.0414
454	363	478	0.9670	1483	1.0607
414	362	447	0.9666	1419	1.0414
340	357	387	0.9648	1304	1.0043
495	351	485	0.9630	1547	1.0792
332	351	375	0.9630	1291	1.0000
389	345	404	0.9610	1381	1.0294
340	344	370	0.9604	1304	1.0043
275	339	324	0.9588	1202	0.9685
674	337	568	0.9582	1828	1.1523
422	337	411	0.9580	1432	1.0453
438	324	400	0.9537	1458	1.0531
462	322	410	0.9530	1496	1.0645
560	318	455	0.9516	1649	1.1072
170	314	243	0.9505	1036	0.9031
495	311	408	0.9495	1547	1.0792

397	298	338	0.9450	1394	1.0334
560	285	384	0.9406	1649	1.1072
251	277	252	0.9380	1164	0.9542
381	240	247	0.9255	1368	1.0253
373	227	226	0.9210	1355	1.0212
495	210	214	0.9154	1547	1.0792
381	174	150	0.9030	1368	1.0253
251	168	151	0.9010	1164	0.9542
105	58	126	0.8640	934	0.8573

Fig 39 Observed TLC Vs TLC from HGB

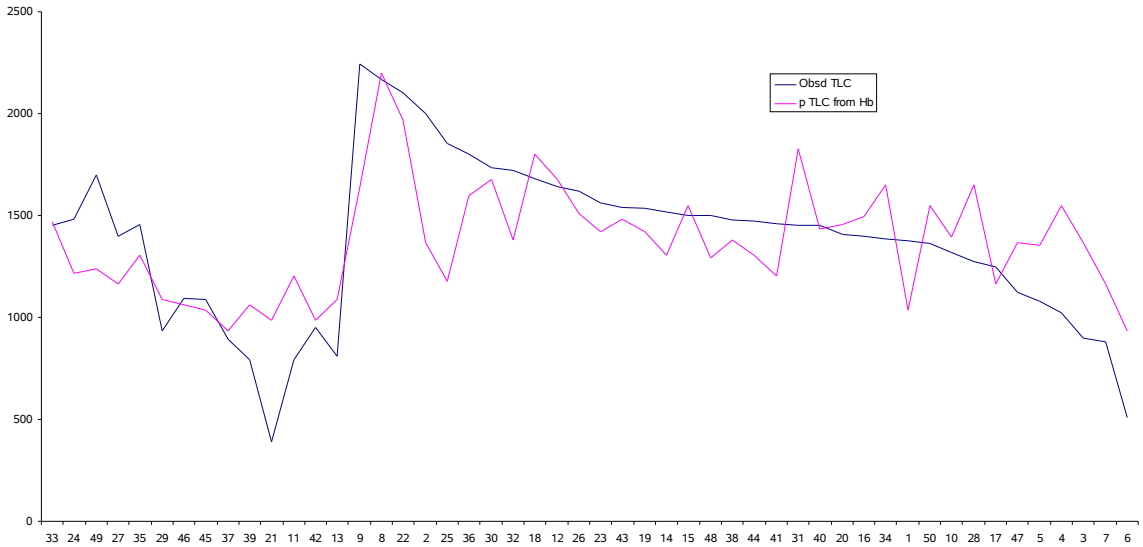


Fig 40 Observed HGB VS HGB From TLC

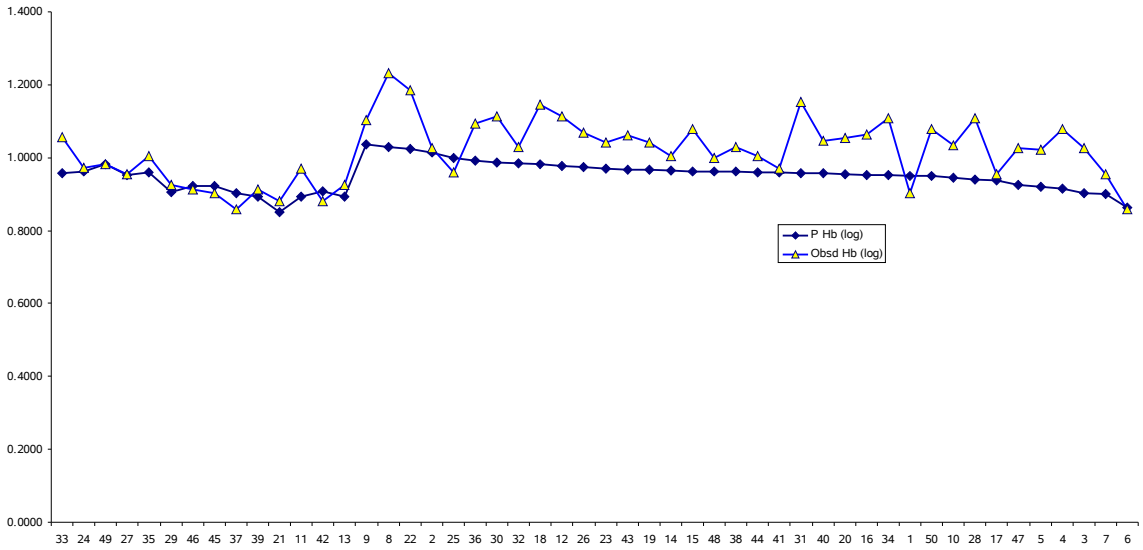
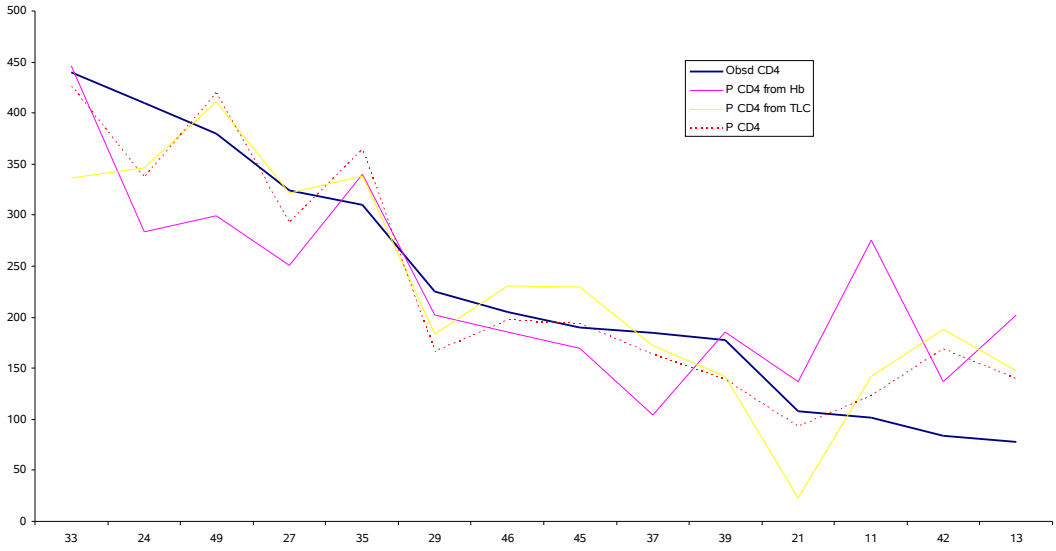


Fig 41 Observed CD4 Vs CD4 from TLC,CD4 from HGB,CD4 from Both



DISCUSSION

Even before researchers discovered that lymphocyte cells consisted of subsets of Bcells and cells derived from the thymus (Tcells), a decrease in the total number of lymphocytes was known to correlate with lack of cellular immunity and predisposition to serve infections in adults and children. (1) As early as 1964, it was recognized that certain childhood infections such as measles temporarily suppress immune function, as reflected in the total lymphocyte count (TLC). As the field of immunology advanced, new laboratory tests, such as mutagen and antigen activation of cells in culture, were used to measure immune function directly. Further analysis identified 2 primary categories of T cells: CD4 cells (also known as “helper T cells”) and CD8 cells.

Soon after HIV was found to be the cause of AIDS, it was shown that the virus binds to receptors on CD4cells, enters the cells, and uses them to create new virus, destroying them in the process. This results in the depletion of CD4 cells and immunodeficiency.

With the increased availability of equipment to performCD4 counts and the knowledge that CD4 cells were the primary target of HIV, the determination of CD4 count became the standard measure of immunodeficiency in adult HIV-infected patients in recourse-rich countries. The relative ease of CD4 cell monitoring also led to its advocacy in treatment guidelines for determining when to start, stop,

or change ART and for deciding when to initiate prophylaxis for opportunistic infections [41]

In resource-limited countries, wide spread and routine use of CD4 count and plasma viral load in the management of HIV infection has not been possible, traditional methods of CD4 count measurement, such as immunophenotyping by flow cytometry or labeling with monoclonal antibodies, require expensive laboratory equipment ad expertise. Even where such facilities have been established at centralized labarotories, specimens typically require processing with in 48 hours of sample collection, which can be difficult to ensure in settings with poor transportation and communication infrastructure .[42]

So many research studies conclude that anemia is frequently experienced by HIV +ve individuals and has been shown to be a strong independent predictor of disease progression and death. HGB measurements is the simplest one for finding anemia, so HGB levels monitor also could be useful as predictor in HIV progression.[40]

WHO-2002 revised guidelines recommends the TLC monitoring as predictor in resource limited setting, and a 2004 study conducted in US evaluated TLC, HGB results as predictors of clinical response to ART. The study found that TLC <1.200 cells/µl ≈ CD_4 <200 cells/µl and H&B <10.6 G/dl ≈ CD_4 < 200 cells/ µl [41]

TLC is Easley obtained from the routine complete blood count (CBC) with differential by percentage lymphocytes by leukocyte

count. In southern India, for example, the cost of a single TLC from a CBC is $1 (US) where as a single CD_4 count by flow cytometry is approximately $30 (US). In India where the average annual income is <$350 and annual per capita spending on health by the government is $133, the cumulative cost of monitoring HAART becomes a significant financial challenge. When computing cost on an annual basis, quarterly monitoring with TLC amounts to only $4 per year while that of CD_4 count testing is $120 per year [42]

Another one least method for classified the HIV patient is symptoms but calculating the persisting of particular symptoms at HIV progression is difficult.

Purpose of this study is to access the capability and clinical utility of TLC change, HGB change to serve as a surrogate marker for CD_4 count in prediction and recommends the HAART initiation criteria.[47]

Of the 50 patients of HIV +ve , 90 % were male ,10 % were female and co morbidity % of study population has given in fig (26) . More than 40 symptoms observed in study population among then 25 were HIV and AIDS related symptoms. Percentage of frequently observed symptoms has given in Table (12).

Among 50 patients 31 were anemic group and 14 patients had CD4 count DATA shown in fig 25,31 and 32.

Patients were sub grouped into 7 different categories based up on their symptoms observed and TLC and HGB Count Decline data shown in Table (17,18) and in fig (26,27,28,29) for each category.

Found the predicted category of CD4 Count measured patients as shown in Table (16) fig (35). Analysed the HGB level, TLC level , Age group , Sex, and category as shown in table(20) .

Correlated the CD4 count of patients in different category with TLC count and HGB count. It showed significant correlation between them figure shown in (41),also we can able to predict the TLC From HGB and vice versa.

Unique symptoms in each category observed from analysis, found that abdominal pain and fever are common symptoms in each category. Symptoms frequency also observed as per our analysis, if one HIV patient come into some symptoms which are easily classified with our model, will easily able to tell that at where he falled in HIV age.

Further HGB and TLC measurement helps to fix that patient into exact category and predict the CD4 form both HGB and TLC. Also can able to predict the TLC by HGB and HGB by TLC is possible by our model. Even though TLC and HGB showed the

significant correlation with CD4 count, the combination of both is a very good predictor.

By our analysis, CD4 count of patient is possible without doing flow cytometry test but just doing TLC and HGB measurements.

SUMMARY & CONCLUSION

HIV infection is associated with numerous co morbidities and opportunistic infection. It affects total immune system of human. Deterioration of immunological parameters is widely used as predictor as well as prognostic marker. Current recommendations of initiating HAART is based on CD_4 count decline. But cost of each test is so high in resource poor settings. So searching of alternative low cost predictors in HIV progression is paramount important in HIV infection for poor resource settings.

Symptoms also important thing in HIV patients. WHO grouped the symptoms into 4 categories and CDC grouped the symptoms into 3 categories, which includes changes of marker trend into this; it will come into seven categories. This integrated model helps us to categorize the HIV patient where they are falling in HIV age and can able to predict the CD_4 count for HIV patients from TLC measurement, Hemoglobin measurement and combined effect of these two measurements. From our analysis correlation of both Hemoglobin and total lymphocyte count with CD4 are significant. WE conclude that TLC and HGB are good predictors for HIV progression in resource limited settings, also combined effect of both is high compared than Individual.

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LIST OF ABBREVATIONS

HIV	-	Human Immunodeficiency Virus
AIDS	-	Acquired Immunodeficiency Syndrome
UNAIDS	-	United Nations Programme on HIV/AIDS
WHO	-	World Health Organisation.
CDC	-	Centre For Disease Control.
Gp41,120	-	Glycoprotein 41,120.
RT	-	Reverse Transcription
IN	-	Integrase Enzyme
VPR	-	Virion Product R
VPU	-	Viral Protein U
TAT	-	Transactivator of Transcription
REV	-	Regulator of Expression Of Virion Proteins
Gag	-	Group Specific Antigen
Nef	-	Negative Effector
TNP	-	Transdominant Negative Protein
EnV	-	Envelop Protein
Mab	-	Monoclonal Anytibody

SFv	-	Intracellular Single Chain Antibody
Si RNA	-	Small Interfering RNA
Sh RNA	-	Short hairpin RNA
NRTI	-	Nucleoside Reverse Transcription inhibitors
NNRTI inhibitors	-	Non Nucleoside Reverse Transcription
PI	-	Protease Inhibitor
HAART	-	Highly Active Antiretoviral Theraphy
HGB	-	Hemoglobin
TLC	-	Total Lymphocyte Count
WBC	-	White Blood Count
OI	-	Opportunistic Infection
VL	-	Virenia Level
CRn	-	C- Reactive Protein
β_2 M	-	Beta 2 Microglobulin
S-neo	-	Serum Neopterin
BMI	-	Body Mass Index

LIST OF FIGURES